DEP-1 RECEPTOR PROTEIN TYROSINE PHOSPHATASE INTERACTING PROTEINS AND RELATED METHODS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Patent Application No. 60/429,746 filed November 26, 2002, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Grant Nos. RO1-GM55989 and T32-CA09311 awarded by the National Institutes of Health. The government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to biomolecules that mediate biological signal transduction in cells, which signals are communicated by phosphorylation and dephosphorylation of cellular proteins for processes such as cellular differentiation, activation, proliferation and survival. More specifically, the invention relates to specific interactions between the protein tyrosine phosphatase known as density enhanced phosphatase-1 (DEP-1) and several distinct cellular proteins, and to related compositions and methods.

Description of the Related Art

Protein tyrosine phosphorylation is an essential element in signal transduction pathways that control fundamental cellular processes including growth and differentiation, cell cycle progression, and cytoskeletal function. Briefly, the binding of hormones, cytokines, growth factors, or other ligands to a cognate receptor protein tyrosine kinase (PTK) triggers

autophosphorylation of tyrosine residues in the receptor itself and phosphorylation of tyrosine residues in the enzyme's target substrates. Within the cell, tyrosine phosphorylation is a reversible process; the phosphorylation state of a particular tyrosine residue in a target substrate is governed by the coordinated action of both PTKs that catalyze phosphorylation and protein tyrosine phosphatases (PTPs) that catalyze dephosphorylation.

The PTPs are a large and diverse family of enzymes found ubiquitously in eukaryotes (Charbonneau and Tonks, *Ann. Rev. Cell Biol.* 8:463-93 (1993)). Structural diversity within the PTP family arises primarily from variation in non-catalytic (potentially regulatory) sequences that are linked to one or more highly conserved catalytic domains. In general, soluble cytoplasmic PTP forms are termed non-receptor PTPs and those with at least one non-catalytic region that traverses the cell membrane are termed receptor-like PTPs (RPTPs).

A variety of non-receptor PTPs have been identified that characteristically possess a single catalytic domain flanked by non-catalytic sequences. Such non-catalytic sequences have been shown to include, among others, sequences homologous to cytoskeletal-associated proteins (Yang et al., *Proc. Natl. Acad. Sci. USA* 88:5949-53 (1991)) or to lipid binding proteins (Gu et al., *Proc. Natl. Acad. Sci. USA* 89:2980-84 (1992)), and/or sequences that mediate association of the enzyme with specific intracellular membranes (Frangioni et al., *Cell* 68:545-60 (1992)), suggesting that subcellular localization may play a significant role in regulation of PTP activity.

Among RPTPs, analysis of non-catalytic domain sequences suggests their involvement in signal transduction mechanisms; however, binding of specific ligands to the extracellular segment of RPTPs has been characterized in only a few instances. For example, homophilic binding has been demonstrated between molecules of PTPμ (Brady-Kalnay et al., *J. Cell. Biol.* 122:961-972 (1993)) *i.e.*, the ligand for PTPμ expressed on a cell surface is another PTPμ molecule on the surface of an adjacent cell. Little is otherwise known about ligands that specifically bind to, and modulate the activity of, the majority of RPTPs.

Many receptor-like PTPs comprise an intracellular carboxyl segment with two catalytic domains, a single transmembrane domain and an extracellular amino terminal

segment (Krueger et al., *EMBO J.* 9:3241-52 (1990)). Subclasses of RPTPs are distinguished from one another on the basis of categories or "types" of extracellular domains (Fischer et al., *Science* 253:401-406 (1991)). Type I RPTPs have a large extracellular domain with multiple glycosylation sites and a conserved cysteine-rich region. CD45 is a typical Type I RPTP. The Type II RPTPs contain at least one amino terminal immunoglobulin (Ig)-like domain adjacent to multiple tandem fibronectin type III (FNIII)-like repeats. Similar repeated FNIII domains, believed to participate in protein-protein interactions, have been identified in receptors for IL2, IL4, IL6, GM-CSF, prolactin, erythropoietin, and growth hormone (Patthy, *Cell* 61:13-14 (1992)). The leukocyte common antigen-related PTP known as LAR exemplifies the Type II RPTP structure (Streuli et al., *J. Exp. Med.* 168:1523-30 (1988)), and, like other Type II RPTPs, contains an extracellular region reminiscent of the NCAM class of cellular adhesion molecules (Edelman and Crossin, *Ann. Rev. Biochem.* 60:155-190 (1991)). The Type III RPTPs, such as HPTPβ (Krueger et al., *EMBO J.* 9:3241-52 (1990)), contain only multiple tandem FNIII repeats in the extracellular domain. The Type IV RPTPs, for example RPTPα (Krueger et al. (1990) *supra*), have relatively short extracellular sequences lacking cysteine residues but containing multiple glycosylation sites. A fifth type of RPTP, exemplified by PTPγ (Barnes et al., *Mol. Cell Biol.* 13:1497-506 (1993)) and PTPζ (Krueger and Saito, *Proc. Natl. Acad. Sci. USA* 89:7417-21 (1992)), is characterized by an extracellular domain containing a 280 amino acid segment that is homologous to carbonic anhydrase (CAH) but lacks essential histidine residues required for reversible hydration of carbon dioxide.

Characteristics shared by both the soluble PTPs and the RPTPs include an absolute specificity for phosphotyrosine residues, a high affinity for substrate proteins, and a specific activity that is one to three orders of magnitude in excess of that of the PTKs *in vitro* (Fischer et al., *Science* 253:401-406 (1991); Tonks, *Curr. Opin. Cell. Biol.* 2:1114-24 (1990)). Supporting a significant physiological role for PTP activity is the observation that treatment of NRK-1 cells with vanadate, a potent inhibitor of PTP activity, resulted in enhanced levels of phosphotyrosine and generation of a transformed cellular morphology (Klarlund, *Cell* 41:707-17 (1985)). This observation implies potential therapeutic value for PTPs and agents that

modulate PTP activity as indirect modifiers of PTK activity and, thus, levels of cellular phosphotyrosine.

Other studies have also highlighted aspects of the physiological importance of PTP activity. For example, mutations in the gene encoding a non-receptor hematopoietic cell protein tyrosine phosphatase, HCP, have been shown to result in severe immune dysfunction characteristic of the motheaten phenotype in mice (Schultz et al., *Cell* 73:1445-54 (1993)). Under normal conditions HCP may act as a suppressor of PTK-induced signaling pathways, for example, the CSF-1 receptor (Schultz et al., *supra*). Some PTP enzymes may be the products of tumor suppressor genes, and their mutation or deletion may contribute to the elevation in cellular phosphotyrosine associated with certain neoplasias (Brown-Shimer et al., *Cancer Res.* 52:478-82 (1992); Wary et al., *Cancer Res.* 53:1498-502 (1993)). Mutations observed in the gene for RPTPγ in murine L cells would be consistent with this hypothesis (Wary et al., *Cancer Res.* 53:1498-502 (1993)). The observation that the receptor-like PTP CD45 is required for normal T cell receptor-induced signaling (Pingel et al., *Cell* 58:1055-65 (1989)) provides evidence implicating PTP activity as a positive mediator of cellular signaling responses. Mice homozygous for a disrupted PTP-1B gene (PTP-1B -/-) exhibited enhanced sensitivity to insulin and resistance to weight gain, relative to controls having functional PTP-1B (Elchebly et al., 1999 *Science* 283:1544).

A variety of ligands trigger the reversible phosphorylation of tyrosyl residues in cellular proteins, a process that underlies the control of such fundamental cellular functions as growth and proliferation, migration and morphogenesis. Tyrosine phosphorylation is regulated by the coordinated action of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Classically it was thought that the PTKs provided the "on -switch" to initiate a physiological response, whereas the PTPs functioned to counteract the PTKs and to return the system to its basal state. However, it has been shown that PTPs may themselves function positively to promote signaling, for example by promoting the dephosphorylation and activation of PTKs, thus coordinating with, rather than antagonizing PTK function (reviewed in (Hermiston et al., *J. Clin. Invest.* 109:9-14 (2002)). A further level of complexity has been introduced with the realization that whether a defined PTP functions positively or negatively

may depend upon the signaling context. Thus, SHP-2 is an activator of signaling through the HGF/SF receptor Met (Maroun et al., *Mol. Cell Biol.* 20:8513-25 (2000)) and the EGF receptor (Bennett et al., *Mol. Cell Biol.* 16:1189-202 (1996)), but is an inhibitor of signaling through the PDGF receptor (Meng et al., *Mol. Cell* 9:387-99 (2002)). Following ligand binding, a receptor PTK may become phosphorylated on multiple tyrosine residues, which serve as docking sites for distinct signaling proteins. The spectrum of such signaling molecules that associate with the PTK will determine the nature of the response that is initiated following ligand stimulation. The possibility exists, therefore, that a PTP may dephosphorylate a particular site in a receptor PTK and thereby determine the signaling outcome of a particular stimulus. Thus, dephosphorylation of receptor PTKs by members of the PTP family may function as a mechanism for regulating the specificity of a signaling event rather than simply as an "off–switch."

Normal cells in culture exhibit contact inhibition of growth, that is, as adjacent cells in a confluent monolayer touch each other, their growth is inhibited (Stoker et al., *Nature* 215:171-72 (1967)). Because PTKs promote cell growth, PTP action may underlie mechanisms of growth inhibition. Density Enhanced PTP-1 (DEP-1) is a Type III receptor PTP whose expression is enhanced as cells approach confluence (Ostman et al., *Proc. Natl. Acad. Sci. USA* 91:9680-84 (1994)). Initially cloned from human cDNA libraries (U.S. Pat. No. 6,114,140; WO95/30008), DEP-1 homologues were subsequently identified in rat and mouse (Kuramochi et al., *FEBS Lett.* 378:7-14 (1996); Borges et al., *Circ. Res.* 79:570-80 (1996)).

DEP-1 comprises an extracellular segment of eight fibronectin type III repeats, a transmembrane domain and a single cytoplasmic PTP domain. Also known as PTP -η (Honda et al., *Blood* 84:4186-94 (1994)) and CD 148 (Palou et al., *Immunol. Lett.* 57:101-103 (1997)), DEP-1 is expressed in a variety of tissues and cell types. There is a growing body of evidence suggesting a role for DEP-1 in the inhibition of cell growth. After vascular injury DEP-1 expression is down regulated in migrating and proliferating rat endothelial cells (Borges et al., *supra*). Attempts have been made to express DEP-1 constitutively in breast cells and macrophages (Keane et al., *Cancer Res.* 56:4236-43 (1996); Osborne et al., *J.*

Leukoc. Biol. 64:692-701 (1998)), however, this inhibited development of stable cell lines, further reinforcing a role for DEP-1 in growth inhibition.

In addition to its role in growth inhibition, DEP-1 has also been implicated in differentiation. The levels of DEP-1 mRNA are increased in various cell lines in response to factors that lead to differentiation (Borges et al., *supra;* Keane et al., *supra*; Zhang et al., *Exp. Cell Res.* 235:62-70 (1997); Martelli et al., *Exp. Cell Res.* 245:195-202 (1998)). Interestingly, in rat thyroid cells the expression of DEP-1 (rPTP -TI) mRNA decreases with increasing levels of transformation (Zhang et al., *supra*; Florio et al., *Endocrinology* 138:3756-63 (1997)). Re-introduction of DEP-1 into the transformed cells leads to reduced growth rates, stabilization of the cyclin-dependent kinase inhibitor $p27^{kip1}$ and partial re-acquisition of a differentiated phenotype (Trapasso et al., *Mol. Cell Biol.* 20:9236-46 (2000)). Loss of DEP-1 expression has also been observed in human thyroid tumors (*id.*). Furthermore, the DEP-1 gene *Ptprj* was identified as a positional candidate for the mouse colon -cancer susceptibility locus Scc1 (Ruivenkamp et al., *Nat. Genet.* 31:295-300 (2002)). Frequent deletions, loss of heterozygosity (LOH) and missense mutations in the human *Ptprj* gene have also been identified in colon, lung and breast cancers (*id.*). Taken together these data indicate that DEP-1 may be a critical factor in controlling cellular growth and transformation.

DEP-1 has recently been shown to localize at cell borders in endothelial cells and its staining pattern overlapped with that of the functional protein VE -cadherin (Takahashi et al., *J. Am. Soc. Nephrol.* 10:2135-45 (1999)). Interestingly, members of the cadherin family of cell -cell adhesion molecules function in the suppression of cell growth and tumor invasion. Junctional components such as β-catenin, however, can also promote cell growth by inducing the transcription of genes involved in proliferation and cancer progression (reviewed in Ben-Ze'ev et al., *Exp. Cell Res.* 261:75-83 (2000)). The growth inhibitory effects of cadherins may involve binding and sequestration of the signaling pool of the catenins (Gottardi et al., *J. Cell Biol.* 153:1049-60 (2001); Stockinger et al., *J. Cell Biol.* 154:1185-96 (2001)). Reversible tyrosine phosphorylation is an important aspect of the regulation of functional integrity and the control of signals emanating from these sites (reviewed in Conacci-Sorrell et al., *J. Clin. Invest.* 109:987-91 (2002)).

Clearly there is a need for the identification of PTPs, PTKs and other components of biological signal transduction pathways that interact with members of these enzyme families, in order to better understand the cellular and molecular mechanisms that govern such processes as cell growth, differentiation and survival in normal and pathological conditions. For instance, determination of the PTKs and PTPs that act upon the components of cell junctions will be important for understanding the regulation of cell morphology and the control of gene expression, events that ultimately influence growth and migration. The present invention contributes to such understanding of the biological signal transduction pathways in which DEP-1 functions by identifying several proteins with which DEP-1 specifically interacts, and offers other related advantages.

BRIEF SUMMARY OF THE INVENTION

It is an aspect of the present invention to provide an isolated complex comprising (a) a DEP-1 polypeptide that is capable of specific association with a DEP-1 substrate polypeptide; and (b) a DEP-1 substrate polypeptide that is in specific association with the DEP-1 polypeptide. In a certain embodiment the DEP-1 polypeptide is selected from (a) a polypeptide which comprises the amino acid sequence set forth in SEQ ID NO:2 (Genbank No. U10886); (b) a polypeptide which comprises the amino acid sequence set forth in SEQ ID NO:3 (positions 997-1337 of SEQ ID NO:2); (c) a polypeptide that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to a nucleic acid molecule which comprises a nucleotide sequence that is a reverse complement of SEQ ID NO:1 (Genbank No. U10886); (d) a truncated DEP-1 polypeptide which comprises at least the amino acid sequence set forth at positions 1205-1245 of SEQ ID NO:2, or a variant thereof; (e) a mutant polypeptide which comprises at least one amino acid substitution in the amino acid sequence set forth in SEQ ID NO:2, wherein the amino acid substitution is selected from a substitution of aspartate at position 1205 and a substitution of cysteine at position 1239; (f) a mutant polypeptide according to (e) wherein aspartate at position 1205 is substituted with alanine; (g) a mutant polypeptide according to (e) wherein cysteine at position 1239 is substituted with serine; (h) a mutant polypeptide which comprises an amino acid sequence as

set forth at positions 997-1337 of SEQ ID NO:2, the mutant polypeptide comprising at least one amino acid substitution that is selected from a substitution of aspartate at position 1205 and a substitution of cysteine at position 1239; (i) a mutant polypeptide according to (h) wherein aspartate at position 1205 is substituted with alanine; (j) a mutant polypeptide according to (h) wherein cysteine at position 1239 is substitute with serine; (k) a polypeptide that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to a nucleic acid molecule which comprises a reverse complement of a nucleotide sequence that encodes a polypeptide selected from any one of (e)-(j); (l) a polypeptide to which binds an antibody that specifically recognizes a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:2; and (m) a polypeptide to which binds an antibody that specifically recognizes a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:3.

In another embodiment the invention provides an isolated complex comprising (a) a DEP-1 polypeptide that is capable of specific dephosphorylation of a DEP-1 substrate polypeptide; and (b) a DEP-1 substrate polypeptide that is in specific association with the DEP-1 polypeptide. According to certain further embodiments, in either of the above described isolated complexes the DEP-1 substrate polypeptide is selected from (a) a polypeptide which comprises the amino acid sequence set forth in any one of SEQ ID NOS:4-6 (SEQ ID NO:4, GenBank Acc. No. P08581; SEQ ID NO:5, Acc. No. AAA59591; SEQ ID NO:6, NM_000245); (b) a polypeptide which comprises a transmembrane domain and a cytoplasmic domain of the polypeptide of (a) as described in Zhu et al. (1994 *Cell Growth Differ.* 5(4):359-366), and which comprises the amino acid sequence set forth in SEQ ID NO:7; (c) at least one $p120^{ctn}$ polypeptide comprising an amino acid sequence as set forth in any one of SEQ ID NOS:8-12 (GenBank Acc. Nos. AF062321, AF062317, AF062319, AF062338, AF062342, respectively); and (d) a Gab1 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 13 (GenBank Acc. No. NM_002039).

In certain other embodiments the invention provides an isolated complex comprising a DEP-1 polypeptide in specific association with a polypeptide selected from (i) a plakoglobin polypeptide comprising an amino acid sequence as set forth in any one of SEQ ID NOS:14-15, and 22 (Acc. No. BC011865, Acc. No. Z68228, Acc. No. NM_021991,

respectively), and (ii) a beta-catenin polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:16 (Acc. No. NM_001904), wherein the DEP-1 polypeptide is selected from the group consisting of members (a)-(m) as described above.

Turning to another aspect of the present invention, a method is provided of identifying an agent that alters interaction of a DEP-1 polypeptide with a DEP-1 substrate polypeptide, comprising (a) exposing, in the absence and presence of a candidate agent, a sample comprising a DEP-1 polypeptide and a DEP-1 substrate polypeptide to conditions sufficient for formation of a complex comprising the DEP-1 polypeptide in specific association with the DEP-1 substrate polypeptide; and (b) comparing a first level of the complex that is formed in the absence of the candidate agent to a second level of the complex that is formed in the presence of the candidate agent, wherein an alteration in the second level relative to the first level indicates that the agent alters interaction between the DEP-1 polypeptide and the DEP-1 substrate polypeptide.

In another embodiment the invention provides a method of identifying an agent that alters dephosphorylation by a DEP-1 polypeptide of a DEP-1 substrate polypeptide, comprising (a) exposing, in the absence and presence of a candidate agent, a sample comprising a DEP-1 polypeptide and a DEP-1 substrate polypeptide to conditions sufficient for (i) formation of a complex comprising the DEP-1 polypeptide in specific association with the DEP-1 substrate polypeptide and (ii) determination of dephosphorylation of the DEP-1 substrate polypeptide; and (b) comparing a first level of DEP-1 substrate polypeptide dephosphorylation in the absence of the candidate agent to a second level of DEP-1 substrate polypeptide dephosphorylation in the presence of the candidate agent, wherein an alteration in the second level relative to the first level indicates that the agent alters dephosphorylation by the DEP-1 polypeptide of the DEP-1 substrate polypeptide. In certain further embodiments of either of the methods just described, the DEP-1 polypeptide is selected from the group consisting of members (a)-(m) as described above. In certain other further embodiments of either of the methods just described, the DEP-1 substrate polypeptide is selected from (a) a polypeptide which comprises the amino acid sequence set forth in any one of SEQ ID NOS:4-6 (SEQ ID NO:4, GenBank Acc. No. P08581; (SEQ ID NO:5, Acc. No. AAA59591; (SEQ ID

NO:6, NM_000245); (b) a polypeptide which comprises a transmembrane domain and a cytoplasmic domain of the polypeptide of (a) as described in Zhu et al. (1994 *Cell Growth Differ.* 5(4):359-366), such polypeptide comprising the amino acid sequence set forth in SEQ ID NO:7, (c) at least one $p120^{ctn}$ polypeptide comprising an amino acid sequence as set forth in any one of SEQ ID NOS:8-12 (GenBank Acc. Nos. AF062321, AF062317, AF062319, AF062338, AF062342, respectively); and (d) a Gab1 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 13 (GenBank Acc. No. NM_002039).

Turning to another aspect, the present invention provides a recombinant expression construct comprising a regulated promoter operably linked to a polynucleotide encoding a DEP-1 polypeptide. In one embodiment the regulated promoter is an inducible promoter, and in another embodiment the regulated promoter is a tightly regulated promoter. In certain embodiments the DEP-1 polypeptide is selected from the group consisting of members (a)-(m) as described above. In a related embodiment the invention provides a host cell comprising the above-described recombinant expression construct, and in another embodiment the invention provides a cell line derived from such a host cell. In certain further embodiments the cell line is an immortal cell line, which in certain still further embodiments may be a cell line derived from a host cell that is a cancer cell, a transformed cell or a malignant cell.

It is another aspect of the invention to provide a method of altering transduction of a biological signal in a cell, comprising introducing into a cell a DEP-1 polypeptide that is capable of specific association with a DEP-1 substrate polypeptide under conditions and for a time sufficient to permit formation of a complex comprising the DEP-1 polypeptide in specific association with the DEP-1 substrate polypeptide, wherein (i) the DEP-1 polypeptide is selected from the group consisting of members (a)-(m) as described above, and wherein (ii) the cell comprises a DEP-1 substrate polypeptide that is selected from (a) a polypeptide which comprises the amino acid sequence set forth in any one of SEQ ID NOS:4-6 (SEQ ID NO:4, GenBank Acc. No. P08581; SEQ ID NO:5, Acc. No. AAA59591; SEQ ID NO:6, NM_000245);(b) a polypeptide which comprises a transmembrane domain and a cytoplasmic domain of the polypeptide of (a) as described in Zhu et al. (1994 *Cell Growth Differ.* 5(4):359-

366), and which comprises the amino acid sequence set forth in SEQ ID NO:7; (c) at least one p120^{ctn} polypeptide comprising an amino acid sequence as set forth in any one of SEQ ID NOS:8-12 (GenBank Acc. Nos. AF062321, AF062317, AF062319, AF062338, AF062342, respectively); and (d) a Gab1 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 13 (GenBank Acc. No. NM_002039). In a further embodiment the step of introducing comprises inducing expression of a polynucleotide that encodes the DEP-1 polypeptide, wherein the polynucleotide is present within the cell. In another embodiment the step of introducing comprises transforming or transfecting the cell with a recombinant expression construct that comprises a polynucleotide that encodes the DEP-1 polypeptide.

In another embodiment the invention provides a method of altering transduction of a biological signal in a cell, comprising contacting a cell with an agent, (i) wherein the cell comprises a DEP-1 polypeptide and a DEP-1 substrate polypeptide, the DEP-1 polypeptide being capable of specific association with the DEP-1 substrate polypeptide to form a complex, (ii) wherein the agent is capable of altering the specific association of the DEP-1 polypeptide with the DEP-1 substrate polypeptide, (iii) wherein the DEP-1 polypeptide is selected from the group consisting of members (a)-(m) as described above, and (iv) wherein the DEP-1 substrate polypeptide is selected from (a) a polypeptide which comprises the amino acid sequence set forth in any one of SEQ ID NOS:4-6 (SEQ ID NO:4, GenBank Acc. No. P08581; SEQ ID NO:5, Acc. No. AAA59591; SEQ ID NO:6, NM_000245); (b) a polypeptide which comprises a transmembrane domain and a cytoplasmic domain of the polypeptide of (a) as described in Zhu et al. (1994 *Cell Growth Differ.* 5(4):359-366), and which comprises the amino acid sequence set forth in SEQ ID NO:7; (c) at least one p120^{ctn} polypeptide comprising an amino acid sequence as set forth in any one of SEQ ID NOS:8-12 (GenBank Acc. Nos. AF062321, AF062317, AF062319, AF062338, AF062342, respectively); and (d) a Gab1 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 13 (GenBank Acc. No. NM_002039).

According to certain further embodiments of either of the above-described methods for altering transduction of a biological signal, formation of the complex results in dephosphorylation of the DEP-1 substrate polypeptide. In a still further embodiment the DEP-

1 substrate polypeptide is selected from (i) a polypeptide which comprises the amino acid sequence set forth in any one of SEQ ID NOS:4-6 (SEQ ID NO:4, GenBank Acc. No. P08581; SEQ ID NO:5, Acc. No. AAA59591; SEQ ID NO:6, NM_000245); and (ii) a polypeptide which comprises a transmembrane domain and a cytoplasmic domain of the polypeptide of (i) as described in Zhu et al. (1994 *Cell Growth Differ.* 5(4):359-366), and which comprises the amino acid sequence set forth in SEQ ID NO:7, and at least one phosphorylated amino acid selected from the amino acid corresponding to position 1349 of SEQ ID NO:4 and the amino acid corresponding to position 1365 of SEQ ID NO:4 is dephosphorylated. In certain other further embodiments of either of the above-described methods for altering transduction of a biological signal, transduction of the biological signal results in altered cell proliferation, differentiation or survival. In certain other further embodiments of either of the above-described methods for altering transduction of a biological signal, transduction of the biological signal results in altered cellular morphogenesis or altered cellular motility.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entireties as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B present immunoblot results of tyrosine phosphorylated proteins trapped by DEP-1(DA) from pervanadate-treated T-47D breast tumor cells. Figure 1A: Immunoblot of tyrosine phosphorylated proteins trapped by DEP-1(DA). T-47D cells were treated with 50 μM pervanadate for 20 minutes prior to lysis. Maltose binding protein (MBP) or MBP.DEP-1 fusion proteins (MBP fused to wildtype DEP-1: MBP.DEP-1; MBP fused to catalytically inactive DEP-1: MBP.DEP-1(CS); and MBP fused to DEP-1 substrate trapping mutant MBP.DEP-1(DA)) were incubated with cell lysates and protein complexes were analyzed by SDS-PAGE and immunoblotting using anti-phosphotyrosine antibodies. An anti-phosphotyrosine immunoprecipitation was also performed on pervanadate treated cell

lysates to illustrate the full complement of tyrosine-phosphorylated proteins (PY IP). Figure 1B: T-47D cells were treated as in Fig. 1A. Cells were lysed with (+) or without (-) 2 mM vanadate. MBP.DEP-1 (wildtype DEP-1 fusion protein) and MBP.DEP-1(CA) fusion protein were pre-incubated with (+) or without (-) 2 mM vanadate and added to cell lysates. Protein complexes were analyzed by SDS-PAGE and immunoblotting using anti-phosphotyrosine antibodies.

Figures 2A and 2B present immunoblots of tyrosine phosphorylated proteins trapped by DEP-1(DA) from pervanadate treated MDA-MB-231 breast tumor cells. Figure 2A: MDA-MB-231 cells were treated with 100 μM pervanadate for 20 minutes prior to lysis. MBP or MBP.DEP-1; MBP.DEP-1(CS); and MBP.DEP-1(DA) fusion proteins were incubated with cell lysates and protein complexes were analyzed by SDS-PAGE and immunoblotting using anti-phosphotyrosine antibodies. Figure 2B. Effects of vanadate on the interaction between tyrosine phosphorylated proteins with the DEP-1(DA) substrate -trapping mutant. MDA-MB-231 cells treated with pervanadate as described above were lysed in lysis buffer with (+) or without (-) 2 mM vanadate. MBP.DEP-1 and MBP.DEP-1(DA) fusion proteins were pre-incubated with (+) or without (-) 2 mM vanadate and added to cell lysates. Protein complexes were analyzed by SDS-PAGE and immunoblotting using anti-phosphotyrosine antibodies.

Figure 3 shows immunoblots that identify tyrosine phosphorylated proteins that interacted with the DEP-1(DA) substrate -trapping mutant. T-47D and T-47 cells ectopically expressing Met (T-47D Met) were treated with 50 μM pervanadate for 20 minutes prior to lysis. MBP.DEP-1 or MBP.DEP-1(DA) fusion proteins were incubated with cell lysates, and protein complexes were analyzed by SDS-PAGE and immunoblotting using antibodies directed towards E-cadherin (E-cad); β-catenin (Beta-cat); plakoglobin (Pg); $p120^{ctn}$ (p120); Met (Met); and Gab 1 (Gab 1). Cell lysate (50 μg) was loaded to confirm the expression and molecular weight of each of the proteins analyzed by immunoblotting (Lysate).

Figures 4A and 4B present immunoblots illustrating co-expression of DEP-1 and Met in 293 cells. 293 cells were transfected with CSF-MET alone (Met) or in combination with wild type (Met + DEP-1) or mutant forms of DEP-1 (Met+DEP-1(CS) or Met+DEP-

1(DA). Figure 4A: Cell lysates were immunoprecipitated with anti-DEP-1 monoclonal antibodies A3 and 143-41 and analyzed by immunoblot (IP DEP-1). Immunoblots probed with the polyclonal anti-DEP-1 antibody CS895A revealed the levels of DEP-1 in the immunoprecipitates (DEP-1) (upper immunoblot). Blots were stripped and re-probed for Met (Met) (lower immunoblot). Figure 4B: Immunoblot analysis of the phosphorylation state of Met in the presence of wild type or mutant forms of DEP-1. Met was immunoprecipitated from the cell lysates using the polyclonal antibody 144 (IP Met). Immunoblots probed with the polyclonal antibody C-12, which is directed to the intracellular segment of Met, revealed the levels of CSF-MET in the immunoprecipitates (Met) (upper immunoblot). Immunoblots were stripped and re-probed with anti-phosphotyrosine antibodies (PY) (lower immunoblot).

Figures 5A-5C present immunoblots demonstrating the effects of expression of DEP-1 on the phosphorylation of Met and on the association of Met with Grb2. Figure 5A: 293 cells were transfected with 20 μg of CSF-MET DNA and 0, 1, 2.5, 5, 10 μg of DEP1 DNA or 10 μg of DEP-1(CS) DNA. Cell lysates (50 μg) were analyzed for the expression levels of DEP-1 (upper immunoblot) and Met (lower immunoblot). Figure 5B: Site-specific dephosphorylation of Met by DEP-1. Met was immunoprecipitated using the polyclonal antibody 144 from the lysates of serum-starved 293 cells transfected as described above. Immunoblots probed with the polyclonal anti-Met antibody C-12 revealed a constant level of Met immunoprecipitated from the cell lysates (MET). This blot was stripped and re-probed with the phospho-specific antibody to Tyr^{1349} in Met (Phospho-Met Y^{1349}). A duplicate blot was probed with anti-phosphotyrosine antibodies to illustrate the total phosphotyrosine content (PY), then sequentially stripped and re-probed with phospho-specific antibodies to examine the phosphorylation status of Tyr^{1230}, Tyr^{1234}, and Tyr^{1235} (Phospho-Met $Y^{1230/34/35}$), and of Tyr^{1365} (phospho-Met Y^{1365}). Figure 5C: Immunoblot analysis of the association of Grb2 with Met. The immunoblots of Met immunoprecipitates described in Figure 5B were probed with an antibody to Grb2 to reveal the level of Grb2 associated with Met (Met IP/Grb2 IB) (upper immunoblot). Cell lysates (50 μg) were also probed with an anti-Grb2 antibody to determine the level of expression of Grb2 in the transfected cells (Lysate) (lower immunoblot).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed in part to the identification of an unexpected set of proteins with which DEP-1 specifically interacts to form heretofore unrecognized molecular complexes that can be isolated, and to related methods. As disclosed herein, DEP-1 specifically interacts with Met polypeptides, with the junctional component catenin $p120^{ctn}$, and with Gab1 polypeptides. Also disclosed herein is the specific interaction of DEP-1 with plakoglobin and with β-catenin.

Isolated complexes provided by the present invention may be used in a variety of contexts relevant to defining and molecularly manipulating biological signal transduction pathways, including defining therapeutic targets and also including, for example, determining additional molecular components of such pathways. In certain preferred embodiments the invention relates to screening assays for agents that alter (*i.e.*, increase or decrease in a statistically significant manner) the interaction of a DEP-1 polypeptide with a DEP-1 substrate polypeptide, for example by altering the association in a complex of DEP-1 with a DEP-1 substrate, and/or by altering the dephosphorylation by DEP-1 of a DEP-1 substrate. Agents so identified will be useful for therapeutic intervention in contexts in which it is desirable to influence biological processes in which DEP-1 complexes play a role, for instance, cell growth or proliferation including cell cycle regulation and contact inhibition of cell growth, cellular differentiation including altered cellular morphogenesis or motility or other cellular activities characterized by alterations in cytoskeletal organization and/or in cellular gene expression, or cell survival including cellular responses to apoptotic stimuli.

Thus, and as described herein, protein complexes according to the present invention may comprise a DEP-1 polypeptide in specific association with a Met polypeptide (*e.g.*, hepatocyte growth factor-receptor, HGF-R, also known as scatter factor receptor, SF-R, GenBank Acc. Nos. P08581 (SEQ ID NO:4), AAA59591 (SEQ IDNO:5), NM_000245 (SEQ ID NO:6); see OMIM (Online Mendelian Inheritance in Man) Acc. No. 164860 (Met proto-oncogene), [online], [retrieved from the Internet on 2002-11-25] Internet <URL:http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>) including a Met-derived polypeptide (SEQ ID NO:7) comprising the transmembrane and cytoplasmic domains of such

a Met polypeptide (Zhu et al., 1994 *Cell Growth Differ.* 5(4):359-366; amino acid positions 938-1408 of GenBank Acc. No. NP_000236; GenBank AAA59591); (*see also* Park et al., *Proc. Natl. Acad. Sci. USA* 84:6379-83 (1987)).

Met induces mitogenic, motogenic and morphogenic responses after ligand activation by recruiting a number of signaling and docking molecules and has been implicated in the phosphorylation of cell junction proteins. Disruption of normal signaling through Met has been implicated in certain cancers (*see, e.g.*, Maulik et al., *Cytokine Growth Factor Rev.* 13:41-59 (2002)). Ligand induced activation of Met by HGF/SF leads to the autophosphorylation of specific tyrosine residues within the PTK. Phosphorylation of Tyr^{1234} and Tyr^{1235} in the activation loop of Met is required for kinase activity, whereas phosphorylation of C-terminal tyrosine residues (Tyr^{1349}, Tyr^{1356}) is required for the recruitment of signaling and adapter molecules including Gab1 (reviewed in Furge et al., *Oncogene* 19:5582-89 (2000))). Additional C-terminal tyrosines such as Tyr^{1365} appear to be important for mediating a morphogenic signal although the identity of proteins that interact with this site is currently unknown (Weidner et al., *Proc. Natl. Acad. Sci. USA* 92:2597-601 (1995); see also Kovalenko et al., *J. Biol. Chem.* 275:14119-23 (2000)). Also described herein is the surprising observation that DEP-1 preferentially dephosphorylates specific tyrosine residues in the C-terminal domain of Met. Without wishing to be bound by theory, such selective dephosphorylation of specific sites in the kinase may provide a mechanism by which DEP-1 attenuates particular signaling events emanating from Met, thus regulating the outcome of cellular responses induced by HGF/SF stimulation.

Met is the prototypic member of a small subfamily of receptor PTKs that includes Ron and the chicken homologue of Ron, Sea. HGF/SF is the ligand for Met, whereas macrophage stimulating protein (MSP) is the ligand for Ron and Sea. Members of this subfamily of PTKs are expressed in a variety of cell types including epithelial, endothelial, and hematopoietic cells. Interestingly, the expression pattern of DEP-1 overlaps with the expression pattern of these receptor PTKs consistent with a possible interaction between these enzymes under physiological conditions.

Following activation by HGF/SF, Met is able to exert a variety of effects by recruiting docking and signaling molecules (*see, e.g.,* Vadnais et al., *J. Biol. Chem.* 277:48342-50 (2002). Epub Oct 07 2002). Phosphorylation of the tyrosine residues in the activation loop of the PTK domain potentiates the intrinsic kinase activity of Met, whereas phosphorylation of the two docking site tyrosine residues (Tyr^{1349}, Tyr^{1356}) allows for the recruitment of adaptor molecules including Grb2, SHC and Gab 1 and signaling enzymes including phosphotidylinositol 3-kinase (PI3K), phospholipase Cγ (PLC-γ), the PTK src, the PTP SHP2, as well as the transcription factor STAT3 (reviewed in Furge et al., *supra*). This multisubstrate docking site sequence is primarily responsible for Met-mediated signal transduction and chimeric receptors containing this sequence can induce mitogenic, motogenic and morphogenic responses similar to Met (Zhu et al., *supra*; Komada et al., *Oncogene* 8:2381-90 (1993); Weidner et al., *J. Cell Biol.* 121:145-54 (1993); Sachs et al., *J. Cell Biol.* 133:1095-107 (1996); *see also, e.g.,* Giordano et al., *Nat. Cell Biol.* 4:720-24 (2002)). Cells expressing Met with mutations at Tyr^{1349} and Tyr^{1356} are unresponsive to HGF/SF stimulation in vitro (Ponzetto et al., *Cell* 77:261-71 (1994)), and transgenic mice with these mutations display a lethal phenotype that resembles the phenotype of mice lacking Met or HGF/SF (Maina et al., *Cell* 87:531-43 (1996)). Modulating the phosphorylation status of the multisubstrate docking site represents an important mechanism for regulating HGF/SF induced cellular responses. As described in greater detail in the Examples herein, DEP-1 preferentially dephosphorylated the docking site residue Tyr^{1349}.

The role of specific adaptor and signaling molecules in transducing Met signals has been studied extensively. The adapter protein Grb2 recruits SOS to activated receptor PTKs to induce Ras-MAP kinase signaling. In Met signaling Ras stimulation is necessary and sufficient to induce proliferation (Ponzetto et al., *J. Biol. Chem.* 271:14119-23 (1996)). Grb2 binds to Met directly at a binding site that contains phosphorylated Tyr^{1356} (Fixman et al., *Oncogene* 10:237-49; Ponzetto et al. (1994), *supra*; Fournier et al., *J. Biol. Chem.* 271:22211-17 (1996)). In addition Grb2 can be recruited to Met via the adapter protein SHC (Pelicci et al., *Oncogene* 10:1631-38 (1995)). After Met activation the adapter molecule Gab 1 is strongly tyrosine phosphorylated and recruited to Met directly through Tyr^{1349} (Weidner et al.,

Nature 384:173-76 (1996)) and indirectly via Grb2 bound to Tyr^{1356} (Bardelli et al., *Oncogene* 15:3103-11 (1997); Nguyen et al., *J. Biol. Chem.* 272:20811-19 (1997); Lock et al., *J. Biol. Chem.* 31536-45 (2000)). Gab 1 can amplify and diversify Met signaling by recruiting additional signaling proteins such as P13K, PLC-y, SHP-2 and the adapter protein Crk. Tyrosine phosphorylation of Gab 1 at specific residues is required for the recruitment of the signaling molecules. Transgenic mice lacking Gab 1 display a lethal phenotype that resembles the phenotype of mice lacking Met or HGF/SF suggesting that Gab 1 is important for Met signaling *in vivo* (*see, e.g.,* Sachs et al., *J. Cell Biol.* 150:1375-84 (2000); s*ee also* Baker et al., *Mol. Cell Biol.* 21:2393-403 (2001)).

As also disclosed herein, protein complexes according to the invention may comprise a DEP-1 polypeptide in specific association with, $p120^{ctn}$, a junctional component catenin polypeptide (*e.g.*, GenBank Acc. Nos. AF062321 (SEQ ID NO:8), AF062317 (SEQ ID NO:9), AF062319 (SEQ ID NO:10), AF062338 (SEQ ID NO:11), AF062342 (SEQ ID NO:12); see OMIM (Online Mendelian Inheritance in Man) Acc. No. 601045 (catenin), [online][retrieved on 2002-11-26]. Retrieved from the Internet:URL:http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>). Also described herein is the association of DEP-1 with Gab1, an adaptor protein (*e.g.*, GenBank Acc. No. NM_002039 (SEQ ID NO:13); see OMIM (Online Mendelian Inheritance in Man) Acc. No. 604439 (Gab1), [online], [retrieved on 2002-11-25]. Retrieved from the Internet:URL:http://www.ncbi.nlm.nih.gov/entrez /query.fcgi?db=OMIM>). Interactions between DEP-1 and plakoglobin (*e.g.*, GenBank Acc. Nos. BC011865 (SEQ ID NO:14), Z68228 (SEQ ID NO:15), NM_021991 (SEQ ID NO: 22; see OMIM (Online Mendelian Inheritance in Man) Acc. No. 173325 (plakoglobin), [online][retrieved from the Internet on 2002-11-25] Retrieved from the Internet:URL:http://www.ncbi.nlm.nih.gov/entrez /query.fcgi?db=OMIM>), and between DEP-1 and β-catenin (*e.g.,* GenBank Acc. No. NM_001904 (SEQ ID NO:16); see OMIM (Online Mendelian Inheritance in Man) Acc. No. 116806 (beta-catenin), [online], [retrieved on 2002-11-25]; retrieved from the Internet:URL:http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>) are also described

herein (*see also* Shibamoto et al., *Cell Adhes. Commun.* 1:295-305 (1994); Holsinger et al., *Oncogene* 21:7067-76 (2002)).

Preferred embodiments of the present invention relate to DEP-1 polypeptides, which include the human DEP-1 polypeptide comprising the amino acid sequence set forth in GenBank Acc. No. U10886 (SEQ ID NO:2), or portions thereof that are capable of specific association with a DEP-1 substrate polypeptide, for instance, a polypeptide comprising the amino acid sequence of positions 997-1337 of SEQ ID NO:2 (as set forth in SEQ ID NO:3), or a truncated polypeptide which comprises at least the amino acid sequence set forth at positions 1205-1245 of SEQ ID NO:2, or a variant thereof as provided herein.

A truncated DEP-1 polypeptide or a variant of such a truncated polypeptide that comprises at least amino acids 1205-1245 of SEQ ID NO:2 may comprise, at either or both of the N-terminus and the C-terminus of the peptide defined by positions 1205-1245 of SEQ ID NO:2, a portion comprising a sequence of an additional 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, or 146 or more of the amino acid residues as set forth in SEQ ID NO:2 that are situated N-terminal to and C-terminal to the fragment defined by positions 1205-1245, and such a polypeptide may further include amino acid substitutions, insertions or deletions at no more than 20%, more preferably no more than 15%, more preferably no more than 10%, still more preferably no more than 5% of the amino acids set forth in SEQ ID NO:2, so long as the polypeptide is capable of specific association with a DEP-1 substrate polypeptide. It should be noted that the DEP-1 polypeptide defined by positions 997-1337 of SEQ ID NO:2 comprises the DEP-1 cytoplasmic domain and that positions 1060-1296 of SEQ ID NO:2 comprise the DEP-1 PTP catalytic domain, which domains may be preferred DEP-1 polypeptides according to certain embodiments of the invention.

In certain embodiments, the present invention thus provides a truncated DEP-1 polypeptide for use in the instant compositions and methods, and in certain other embodiments the invention provides nucleic acids encoding such a truncated DEP-1 polypeptide. A truncated molecule may be any molecule that comprises less than a full-length version of the molecule. Truncated molecules provided by the present invention may include truncated biological polymers, and in preferred embodiments of the invention such truncated molecules may be truncated nucleic acid molecules or truncated polypeptides. Truncated nucleic acid molecules have less than the full-length nucleotide sequence of a known or described nucleic acid molecule. Such a known or described nucleic acid molecule may be a naturally occurring, a synthetic, or a recombinant nucleic acid molecule, so long as one skilled in the art would regard it as a full-length molecule. Thus, for example, truncated nucleic acid molecules that correspond to a gene sequence contain less than the full length gene where the gene comprises coding and non-coding sequences, promoters, enhancers and other regulatory sequences, flanking sequences and the like, and other functional and non-functional sequences that are recognized as part of the gene. In another example, truncated nucleic acid molecules that correspond to a mRNA sequence contain less than the full length mRNA transcript, which may include various translated and non-translated regions as well as other functional and non-functional sequences.

In other preferred embodiments, truncated molecules are polypeptides that comprise less than the full-length amino acid sequence of a particular protein or polypeptide component, for instance, a DEP-1 polypeptide or a DEP-1 substrate polypeptide as provided herein. As used herein "deletion" has its common meaning as understood by those familiar with the art, and may refer to molecules that lack one or more of a portion of a sequence from either terminus or from a non-terminal region, relative to a corresponding full-length molecule, for example, as in the case of truncated molecules provided herein. Truncated molecules that are linear biological polymers such as nucleic acid molecules or polypeptides may have one or more of a deletion from either terminus of the molecule and/or a deletion from a non-terminal region of the molecule. Such deletions may be deletions of 1-1500 contiguous nucleotide or amino acid residues, preferably 1-500 contiguous nucleotide or amino acid residues and more

preferably 1-300 contiguous nucleotide or amino acid residues, including deletions of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31-40, 41-50, 51-74, 75-100, 101-150, 151-200, 201-250 or 251-299 contiguous nucleotide or amino acid residues. In certain particularly preferred embodiments truncated nucleic acid molecules may have at least one deletion of approximately 270-330 contiguous nucleotides. In certain other particularly preferred embodiments truncated polypeptide molecules may have at least one deletion of 40-140 contiguous amino acids.

A DEP-1 polypeptide for use according to certain embodiments of the present invention comprises a polypeptide that binds to an antibody which specifically recognizes (*e.g.*, binds to) a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:2. According to certain other embodiments a DEP-1 polypeptide comprises a polypeptide that binds to an antibody which specifically recognizes (*e.g.*, binds to) a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:3.

Therefore, also contemplated by the present invention is the use according to certain embodiments of an antibody that specifically binds to a DEP-1 polypeptide, or the use of other molecules that specifically bind to a DEP-1 polypeptide and which may include peptides, polypeptides, and other non-peptide molecules that specifically bind to a DEP-1 polypeptide and in particularly preferred embodiments, to a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:3. As used herein, a molecule is said to "specifically bind" to a DEP-1 peptide or polypeptide if it reacts at a detectable level with the DEP-1 peptide or polypeptide, but does not react detectably with peptides containing an unrelated sequence or a sequence of a different phosphatase. Preferred binding molecules include antibodies, which may be, for example, polyclonal, monoclonal, single chain, chimeric, anti-idiotypic, or CDR-grafted immunoglobulins, or fragments thereof, such as proteolytically generated or recombinantly produced immunoglobulin $F(ab')_2$, Fab, Fv, and Fd fragments. Binding properties of an antibody to a DEP-1 may generally be assessed using immunodetection methods including, for example, an enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, immunoblotting and the like, which may be readily performed by those having ordinary skill in the art. In certain preferred embodiments, the invention

method may relate to isolating a DEP-1 polypeptide with an antibody that specifically binds to the phosphatase; such embodiments may include without limitation methodologies for immuno-isolation (*e.g.*, immunoprecipitation, immunoaffinity chromatography) and/or immunodetection (*e.g.*, western blot).

Methods well known in the art may be used to generate antibodies, polyclonal antisera or monoclonal antibodies, that are specific for DEP-1; a number of DEP-1-specific antibodies are also commercially available. As used herein, an antibody is said to be "immunospecific" or to "specifically bind" a DEP-1 polypeptide if it reacts at a detectable level with DEP-1, preferably with an affinity constant, K_a, of greater than or equal to about 10^4 M^{-1}, more preferably of greater than or equal to about 10^5 M^{-1}, more preferably of greater than or equal to about 10^6 M^{-1}, and still more preferably of greater than or equal to about 10^7 M^{-1}. Affinity of an antibody for its cognate antigen is also commonly expressed as a dissociation constant K_D, and an anti-DEP-1 antibody specifically binds to DEP-1 if it binds with a K_D of less than or equal to 10^{-4} M, more preferably less than or equal to about 10^{-5} M, more preferably less than or equal to about 10^{-6} M, still more preferably less than or equal to 10^{-7} M, and still more preferably less than or equal to 10^{-8} M. Affinities of binding partners or antibodies can be readily determined using conventional techniques, for example, those described by Scatchard et al. (*Ann. N.Y. Acad. Sci. USA 51*:660 (1949)) or by surface plasmon resonance (BIAcore, Biosensor, Piscataway, NJ) *(see, e.g.,* Wolff et al., *Cancer Res. 53*:2560-2565 (1993)).

Antibodies may generally be prepared by any of a variety of techniques known to those having ordinary skill in the art. *See, e.g.,* Harlow et al., *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory (1988). In one such technique, an animal is immunized with an antigen to generate polyclonal antisera. Suitable animals include, for example, rabbits, sheep, goats, pigs, cattle, and may also include smaller mammalian species, such as mice, rats, and hamsters, or other species. An immunogen may be comprised of cells expressing DEP-1, purified or partially purified DEP-1 polypeptides or variants or fragments (*e.g.*, peptides) thereof, or DEP-1 peptides. PTP peptides may be generated by proteolytic cleavage or may be chemically synthesized. For instance, nucleic acid sequences encoding

DEP-1 polypeptides are provided herein, such that those skilled in the art may routinely prepare these polypeptides for use as immunogens. Polypeptides or peptides useful for immunization may also be selected by analyzing the primary, secondary, and tertiary structure of DEP-1 according to methods known to those skilled in the art, in order to determine amino acid sequences more likely to generate an antigenic response in a host animal. *See, e.g.*, Novotny, 1991 *Mol. Immunol. 28*:201-207; Berzofsky, 1985 *Science* 229:932-40.

Certain embodiments of the invention contemplate mutant DEP-1 polypeptides, including those that comprise at least one amino acid substitution in the amino acid sequence set forth in SEQ ID NO:2, which in certain preferred embodiments comprises substitution of the aspartate at position 1205 of SEQ ID NO:2 and/or substitution of the cysteine at position 1239 of SEQ ID NO:2. In certain particularly preferred embodiments aspartate at position 1205 is substituted with alanine. In certain particularly preferred embodiments cysteine at position 1239 is substituted with serine.

Portions of two polypeptide sequences (*e.g.*, DEP-1 polypeptides, DEP-1 substrate polypeptides or other DEP-1 interacting or DEP-1 associating polypeptides) are regarded as "corresponding" amino acid sequences, regions, fragments or the like, based on a convention of numbering one sequence according to amino acid position number, and then aligning the sequence to be compared in a manner that maximizes the number of amino acids that match or that are conserved residues, for example, that remain polar (*e.g.*, D, E, K, R, H, S, T, N, Q), hydrophobic (*e.g.*, A, P, V, L, I, M, F, W, Y) or neutral (e.g., C, G) residues at each position. Similarly, a DNA sequence encoding a candidate polypeptide that is to be mutated as provided herein, or a portion, region, fragment or the like, may correspond to a known wildtype polypeptide-encoding DNA sequence according to a convention for numbering nucleic acid sequence positions in the known wildtype DNA sequence, whereby the candidate DNA sequence is aligned with the known DNA such that at least 70%, preferably at least 80% and more preferably at least 90% of the nucleotides in a given sequence of at least 20 consecutive nucleotides of a sequence are identical. In certain preferred embodiments, a candidate DNA sequence is greater than 95% identical to a corresponding known DNA sequence. In certain particularly preferred embodiments, a portion, region or fragment of a

candidate DNA sequence is identical to a corresponding known DNA sequence. As is well known in the art, an individual whose DNA contains no irregularities (*e.g.*, a common or prevalent form) in a particular gene responsible for a given trait may be said to possess a wildtype genetic complement (genotype) for that gene, while the presence of irregularities known as mutations in the DNA for the gene, for example, substitutions, insertions or deletions of one or more nucleotides, indicates a mutated or mutant genotype.

As noted above, in certain embodiments of the present invention a substrate trapping mutant PTP is provided in which the catalytic domain invariant aspartate and, optionally, at least one tyrosine residue are replaced, as provided in U.S. Pat. Nos. 5,912,138, 5,951,979, and PCT/US00/14211 (WO 00/75339), all incorporated by reference. Preferably the tyrosine residue that is replaced is located in the PTP catalytic domain, which refers to the approximately 250 amino acid region that is highly conserved among the various PTPs, as noted above (*see also, e.g.*, Barford, 1998 *Ann. Rev. Biophys. Biomol. Struct.* 27:133; Jia, 1997 *Biochem. Cell Biol.* 75:17; Van Vactor et al., 1998 *Curr. Opin Genet. Devel.* 8:112). More preferably, the tyrosine residue is located in a PTP active site, which refers to the region within the PTP catalytic domain that contains the PTP signature motif and which also includes those amino acids that form the PTP binding site pocket or "cradle" for substrate binding and dephosphorylation, further including the invariant aspartate-containing loop (when present) and adjacent peptide backbone sequences that contribute to substrate recognition and catalysis (see, *e.g.*, Jia, 1997).

Within the conserved catalytic domain is a unique signature sequence motif, CX_5R (SEQ ID NO: 17), that is invariant among all PTPs. In a majority of PTPs, an 11 amino acid conserved sequence ([I/V]HCXAGXXR[S/T]G (SEQ ID NO: 18)) containing the signature sequence motif is found. The cysteine residue in this motif is invariant in members of the family and is essential for catalysis of the phosphotyrosine dephosphorylation reaction. It functions as a nucleophile to attack the phosphate moiety present on a phosphotyrosine residue of the incoming substrate. In certain embodiments the cysteine residue that is present in the PTP signature catalytic motif CX_5R (SEQ ID NO: 17) is modified to yield a catalytically inactive PTP; typically the cysteine residue is replaced with serine as described, for example,

by Sun et al. (1993 *Cell 75*:487-493), but other substitutions may also be made. If the cysteine residue is altered by site-directed mutagenesis to serine (*e.g.,* in cysteine-to-serine or "CS" mutants) or alanine (*e.g.,* cysteine-to-alanine or "CA" mutants), the resulting PTP is catalytically deficient but retains the ability to complex with, or bind, its substrate, at least *in vitro*.

Identification of the catalytic domain invariant aspartate residue in PTP sequences other than those disclosed in Barford et al. (1995), or of the cysteine residue that is present in the PTP signature catalytic motif CX_5R (SEQ ID NO: 17), may be achieved by comparing sequences using computer algorithms well known to those having ordinary skill in the art, such as GENEWORKS, Align or the BLAST algorithm (Altschul, *J. Mol. Biol. 219*:555-565, 1991; Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA 89*:10915-10919, 1992), which is available at the NCBI website. Therefore it should be recognized that mutant DEP-1 polypeptides other than those specifically described herein can readily be made by aligning the amino acid sequence of a DEP-1 catalytic domain with the amino acid sequence of DEP-1 polypeptides that are described herein (including those provided by the cited references), identifying the catalytic domain invariant aspartate residue and, optionally, at least one tyrosine residue, and changing these residues, for example by site-directed mutagenesis of DNA encoding the PTP.

Accordingly, certain embodiments of the invention pertain in part to PTPs in which the invariant aspartate residue is replaced with an amino acid which does not cause significant alteration of the Km of the enzyme (that is, does not cause a statistically significant increase or decrease of the Km) but which results in a reduction in Kcat to less than 1 per minute (less than 1 min^{-1}). That is, replacement of the wildtype aspartate residue results in a reduction of Kcat such that the Kcat of the substrate trapping mutant is less than 1 per minute, which is a reduction in Kcat compared with the wildtype PTP. As understood by persons skilled in the art, the Michaelis constant Km is a term that indicates a measure of the substrate concentration required for effective catalysis to occur and is the substrate concentration at which the reaction is occurring at one-half its maximal rate (1/2 Vmax). The Kcat of an enzyme provides a direct measure of the catalytic production of product under optimum

conditions (particularly, saturated enzyme). The reciprocal of Kcat is often referred to as the time required by an enzyme to "turn over" one substrate molecule, and Kcat is sometimes called the turnover number. Vmax and Kcat are directly proportional; therefore, if, for example, Kcat of a substrate trapping mutant is reduced by 10^4 compared to the Kcat of the wildtype enzyme, Vmax is also decreased by a factor of 10^4. These substrate trapping mutant PTPs retain the ability to form a complex with, or bind to, their tyrosine phosphorylated substrates, but are catalytically attenuated (*i.e.,* a substrate trapping mutant PTP retains a similar Km to that of the corresponding wildtype PTP, but has a Vmax which is reduced by a factor of at least 10^2-10^5 relative to the wildtype enzyme, depending on the activity of the wildtype enzyme relative to a Kcat of less than 1 min^{-1}). This attenuation includes catalytic activity that is either reduced or abolished relative to the wildtype PTP. For example, the invariant aspartate residue can be changed or mutated to an alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, lysine, arginine or histidine.

Without wishing to be bound by theory, such a substrate trapping mutant PTP may reduce the activity of the corresponding wildtype PTP by forming a complex with the tyrosine phosphorylated protein substrate of the wildtype PTP, thereby rendering the substrate unavailable for catalytic dephosphorylation by the wildtype enzyme. The substrate trapping mutant PTP thus binds to the phosphoprotein substrate without dephosphorylating it (or catalyzing dephosphorylation at a greatly reduced rate), thereby blocking the activity of the dephosphorylated protein substrate and reducing its downstream effects. As used herein, "reducing" includes both reduction and complete abolishment of one or more activities or functions of the phosphorylated protein substrate.

The preferred substrate trapping mutant PTPs described herein, in which the invariant aspartate residue is replaced with an amino acid which does not cause significant alteration of the Km of the enzyme but which results in a reduction in Kcat to less than 1 per minute (less than 1 min^{-1}), and/or in which at least one tyrosine residue is replaced with an amino acid that is not capable of being phosphorylated, may additionally or alternatively comprise other mutations. In particularly preferred embodiments, such additional mutations

relate to substitutions, insertions or deletions (most preferably substitutions) that assist in stabilizing the PTP/substrate complex. For example, mutation of the serine/threonine residue in the signature motif to an alanine residue (S/T→A mutant) may change the rate-determining step of the PTP-mediated substrate dephosphorylation reaction. For the unmodified PTP, formation of the transition state may be rate-limiting, whereas in the case of the S/T→A mutant, the breakdown of the transition state may become rate-limiting, thereby stabilizing the PTP/substrate complex. Such mutations may be valuably combined with the replacement of the PTP catalytic domain invariant aspartate residue and the replacement of PTP tyrosine as provided herein, for example, with regard to stabilizing the PTP-substrate complex and facilitating its isolation. As another example, substitution of any one or more other amino acids present in the wildtype PTP that are capable of being phosphorylated as provided herein (*e.g.,* serine, threonine, tyrosine) with an amino acid that is not capable of being phosphorylated may be desirable, with regard to the stability of a PTP-substrate complex.

As noted above, in certain embodiments the present invention relates to substrate trapping mutant PTPs in which catalytic domain invariant aspartate and at least one tyrosine residue are replaced, wherein the tyrosine is replaced with an amino acid that is not capable of being phosphorylated. The amino acid that is not capable of being phosphorylated may, in preferred embodiments, be alanine, cysteine, aspartic acid, glutamine, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, arginine, valine or tryptophan. The desirability of the tyrosine replacement derives from the observation that under certain conditions *in vivo*, a PTP enzyme may itself undergo tyrosine phosphorylation in a manner that can alter interactions between the PTP and other molecules, including PTP substrates (*e.g.*, WO 00/75339).

DEP-1 substrate polypeptides as provided herein include any naturally or non-naturally tyrosine-phosphorylated peptide, polypeptide or protein that can specifically associate with, bind to and/or be dephosphorylated by a DEP-1 polypeptide as provided herein. Thus, in addition to substitution of DEP-1 invariant aspartate (*e.g.*, position 1205 of SEQ ID NO:2 or a corresponding amino acid in a truncated DEP-1 polypeptide) and/or of DEP-1 CX_5R cysteine (*e.g.*, position 1239 of SEQ ID NO:2 or a corresponding amino acid in a truncated

DEP-1 polypeptide), replacement of a tyrosine residue found in the wildtype DEP-1 amino acid sequence with another amino acid as provided herein may stabilize the complex formed by the mutant DEP-1 polypeptide and the DEP-1 substrate polypeptide such that the amount of complex that is present increases and/or the affinity of the mutant DEP-1 for the substrate increases, relative to the amount of complex formed using a DEP-1 polypeptide in which the tyrosine residue is not replaced.

As noted above, in certain embodiments the present invention exploits mutant DEP-1 polypeptides described herein (*e.g.*, substrate trapping mutants) to provide a method of screening for an agent that alters (*i.e.*, increases or decreases in a statistically significant manner relative to an appropriate control as will be known to the ordinarily skilled artisan) an activity or interaction (*e.g.*, binding to form a complex or catalytic dephosphorylation) between a tyrosine phosphorylated protein that is a substrate of a wildtype DEP-1 and the DEP-1 polypeptide, which in preferred embodiments will be a method of screening for an inhibitor of an interaction between a DEP-1 polypeptide and a DEP-1 substrate polypeptide.

According to this aspect of the invention, a sample comprising at least one tyrosine phosphorylated protein (*e.g.*, a DEP-1 substrate polypeptide as described herein such as a Met polypeptide, a $p120^{ctn}$ polypeptide or a Gab1 polypeptide that is capable of specific association with, and optionally dephosphorylation by, an appropriate DEP-1 polypeptide) or at least one polypeptide (*e.g.*, a plakoglobin or a β-catenin polypeptide as provided herein) that is capable of specific association with a DEP-1 polypeptide, is combined with at least one DEP-1 polypeptide, for example a substrate trapping mutant DEP-1 as provided herein, and the presence or absence of a complex comprising the substrate and the DEP-1 polypeptide is determined.

The binding interaction between a DEP-1 polypeptide and a DEP-1 substrate polypeptide or other interacting polypeptide may thus result in the formation of a complex, which refers to the affinity interaction of the DEP-1 and the DEP-1 substrate. A complex may include a signaling complex, which refers to any complex that, by virtue of its formation, its stable association and/or its dissociation directly or indirectly provides a biological signal. Such signals may include, for example by way of illustration and not limitation, intracellular

and/or intercellular events that lead to molecular binding, covalent or non-covalent modification of molecular structure, gene expression, genetic recombination, genetic integration, nucleic acid synthesis or subcellular particle assembly, and may also include endocytic, phagocytic, nucleolytic, proteolytic, lipolytic, hydrolytic, catalytic, or other regulatory events.

Determination of the presence of a stable complex between a DEP-1 polypeptide and a DEP-1 substrate polypeptide (or other DEP-1-interacting polypeptide) refers to the use of any methodology known in the art for demonstrating an intermolecular interaction between a PTP and a PTP substrate according to the present disclosure. Such methodologies may include, by way of illustration and not limitation, co-purification, co-precipitation, co-immunoprecipitation, radiometric or fluorimetric assays, western immunoblot analyses, affinity capture including affinity techniques such as solid-phase ligand-counterligand sorbent techniques, affinity chromatography and surface affinity plasmon resonance, and the like. For these and other useful affinity techniques, see, for example, Scopes, R.K., *Protein Purification: Principles and Practice*, 1987, Springer-Verlag, NY; Weir, D.M., *Handbook of Experimental Immunology*, 1986, Blackwell Scientific, Boston; and Hermanson, G.T. et al., *Immobilized Affinity Ligand Techniques*, 1992, Academic Press, Inc., California; which are hereby incorporated by reference in their entireties, for details regarding techniques for isolating and characterizing complexes, including affinity techniques. A DEP-1 polypeptide may interact with a DEP-1 substrate polypeptide, or with another DEP-1-interacting polypeptide, via specific binding if the DEP-1 binds the substrate (or interacting polypeptide) with a K_a of greater than or equal to about 10^4 M^{-1}, preferably of greater than or equal to about 10^5 M^{-1}, more preferably of greater than or equal to about 10^6 M^{-1} and still more preferably of greater than or equal to about 10^7 M^{-1} to 10^9 M^{-1}. Affinities of binding partners such as a DEP-1 polypeptide and a DEP-1 substrate polypeptide can be readily determined using conventional techniques, for example by surface plasmon resonance and those described by Scatchard et al., *Ann. N.Y. Acad. Sci. 51*:660 (1949). Similarly, as described above for the affinity of an antibody and its cognate antigen, affinity of DEP-1 for its substrate may be expressed as a dissociation constant K_D, and DEP-1 specifically binds to a DEP-1 substrate if it

binds with a K_D of less than or equal to 10^{-4} M, more preferably less than or equal to about 10^{-5} M, more preferably less than or equal to about 10^{-6} M, still more preferably less than or equal to 10^{-7} M, and still more preferably less than or equal to 10^{-8} M.

Modification of DNA may be performed by a variety of methods, including site-specific or site-directed mutagenesis of DNA encoding the PTP (*e.g.*, a DEP-1 polypeptide as provided herein) and the use of DNA amplification methods using primers to introduce and amplify alterations in the DNA template, such as PCR splicing by overlap extension (SOE). Site-directed mutagenesis is typically effected using a phage vector that has single- and double-stranded forms, such as M13 phage vectors, which are well-known and commercially available. Other suitable vectors that contain a single-stranded phage origin of replication may be used (*see, e.g.*, Veira et al., *Meth. Enzymol. 15*:3, 1987). In general, site-directed mutagenesis is performed by preparing a single-stranded vector that encodes the protein of interest (*e.g.*, a member of the PTP family). An oligonucleotide primer that contains the desired mutation within a region of homology to the DNA in the single-stranded vector is annealed to the vector followed by addition of a DNA polymerase, such as *E. coli* DNA polymerase I (Klenow fragment), which uses the double stranded region as a primer to produce a heteroduplex in which one strand encodes the altered sequence and the other the original sequence. Additional disclosure relating to site-directed mutagenesis may be found, for example, in Kunkel et al. (*Methods in Enzymol. 154*:367, 1987); and in U.S. Patent Nos. 4,518,584 and 4,737,462. The heteroduplex is introduced into appropriate bacterial cells, and clones that include the desired mutation are selected. The resulting altered DNA molecules may be expressed recombinantly in appropriate host cells to produce the modified protein.

Specific substitutions of individual amino acids through introduction of site-directed mutations are well-known and may be made according to methodologies with which those having ordinary skill in the art will be familiar. The effects on catalytic activity of the resulting mutant DEP-1 polypeptide may be determined empirically merely by testing the resulting modified protein for the preservation of the Km and reduction of Kcat to less than 1 per minute as provided herein and as previously disclosed (*e.g.*, WO98/04712; Flint et al., 1997 *Proc. Nat. Acad. Sci.* 94:1680). The effects on the ability to tyrosine phosphorylate the

resulting mutant PTP molecule can also be determined empirically merely by testing such a mutant for the presence of phosphotyrosine, as also provided herein, for example, following exposure of the mutant to conditions *in vitro* or *in vivo* where it may act as a PTK acceptor.

Although the specific examples of mutant DEP-1 polypeptides described herein are DA (aspartate to alanine) mutants, YF (tyrosine to phenylalanine) mutants, CS mutants and combinations thereof, it will be understood that the subject invention substrate trapping mutant DEP-1 polypeptides are not limited to these amino acid substitutions. The invariant aspartate residue can be changed, for example by site-directed mutagenesis, to any amino acid that does not cause significant alteration of the Km of the enzyme but which results in a reduction in Kcat to less than 1 per minute (less than 1 min^{-1}). For example, the invariant aspartate residue can be changed or mutated to an alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, lysine, arginine or histidine, or other natural or non-natural amino acids known in the art including derivatives, variants and the like. Similarly, substitution of at least one tyrosine residue may be with any amino acid that is not capable of being phosphorylated (*i.e.*, stable, covalent modification of an amino acid side chain at a hydroxyl with a phosphate group), for example alanine, cysteine, aspartic acid, glutamine, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, arginine, valine or tryptophan, or other natural or non-natural amino acids known in the art including derivatives, variants and the like.

The nucleic acids of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. A nucleic acid molecule encoding a DEP-1 polypeptide, or a substrate trapping mutant DEP-1 in which the wildtype protein tyrosine phosphatase catalytic domain invariant aspartate residue is replaced with an amino acid which does not cause significant alteration of the Km of the enzyme but which results in a reduction in Kcat to less than 1 per minute, and in which at least one wildtype tyrosine residue is replaced with an amino acid that is not capable of being phosphorylated, may be identical to the coding

sequence known in the art for DEP-1 (*e.g.*, SEQ ID NO:1), or may be a different coding sequence, which, as a result of the redundancy or degeneracy of the genetic code, encodes the same PTP.

According to certain embodiments of the present invention, a DEP-1 polypeptide may be encoded by a polynucleotide that hybridizes under moderately stringent conditions to a nucleic acid molecule which comprises a nucleotide sequence that is a reverse complement of SEQ ID NO:1. Suitable moderately stringent conditions include, for example, prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-70°C, 5 X SSC, for 1-16 hours (*e.g.*, overnight); followed by washing once or twice at 22-65°C for 20-40 minutes with one or more each of 2X, 0.5X and 0.2X SSC containing 0.05-0.1% SDS. By way of example, conditions for a moderately stringent wash may include 0.2X SSC and 0.1% SDS for 15 minutes at 42°C. For additional stringency, conditions may include a wash in 0.1X SSC and 0.1% SDS at 50-70 °C for 15-40 minutes. As known to those having ordinary skill in the art, variations in stringency of hybridization conditions may be achieved by altering the time, temperature and/or concentration of the solutions used for prehybridization, hybridization and wash steps, and suitable conditions may also depend in part on the particular nucleotide sequences, length, and base composition of the probe used, and of the blotted, proband nucleic acid sample.

The present invention further relates to variants of the herein described nucleic acids which encode fragments, analogs and derivatives of a DEP-1 polypeptide, including a mutated DEP-1 such as a substrate trapping mutant DEP-1 or a catalytically inactive DEP-1. The variants of the nucleic acids encoding DEP-1 polypeptides may be naturally occurring allelic variants of the nucleic acids or non-naturally occurring variants. As is known in the art, an allelic variant is an alternate form of a nucleic acid sequence which may have at least one of a substitution, a deletion or an addition of one or more nucleotides, any of which does not substantially alter the function of the encoded PTP polypeptide.

Equivalent DNA constructs that encode various additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences not needed for biological activity are also encompassed by the invention. For example, sequences

encoding Cys residues that are not essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, preventing formation of incorrect intramolecular disulfide bridges upon renaturation. Other equivalents can be prepared by modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

The present invention further relates to DEP-1 polypeptides including substrate trapping mutant PTPs, and in particular to methods for producing recombinant DEP polypeptides by culturing host cells containing DEP-1 expression constructs, and to isolated recombinant DEP-1 polypeptides. The polypeptides and nucleic acids of the present invention are preferably provided in an isolated form, and in certain preferred embodiments are purified to homogeneity. The terms "fragment," "derivative" and "analog" when referring to DEP-1 polypeptides or fusion proteins, including substrate trapping mutant DEP-1 polypeptides, refers to any DEP-1 polypeptide or fusion protein that retains essentially the same biological function or activity as such polypeptide (*e.g.*, ability to specifically associate with a DEP-1 substrate polypeptide or other DEP-1 associating polypeptide). Thus, an analog includes a proprotein that can be activated by cleavage of the proprotein portion to produce an active DEP-1 polypeptide. The polypeptides of the present invention may be recombinant polypeptides or synthetic polypeptides, and are preferably recombinant polypeptides.

A fragment, derivative or analog of a DEP-1 polypeptide or fusion protein, including substrate trapping mutant DEP-1, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue), and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues

includes a substituent group, or (iii) one in which the DEP-1 polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (*e.g.*, polyethylene glycol), or (iv) one in which additional amino acids are fused to the DEP-1 polypeptide, including amino acids that are employed for purification of the DEP-1 polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

These and related properties of a DEP-1 polypeptide may be advantageously engineered into such a polypeptide where a particular use is contemplated. For example, according to certain embodiments of the invention there is provided a method of altering transduction of a biological signal in a cell comprising introducing into a cell a DEP-1 polypeptide that is capable of specific association with a DEP-1 substrate polypeptide under conditions and for a time sufficient to permit formation of a complex comprising the DEP-1 polypeptide in specific association with the substrate. Accordingly, related embodiments of the invention contemplate DEP-1 polypeptides that are fusion proteins comprising a truncated DEP-1 polypeptide domain as provided herein that is capable of specific association with a DEP-1 substrate, fused to a domain selected to deliver the polypeptide into a cell. A number of such polypeptide domains are known to the art (*e.g.*, Mahat et al., 1999 *Curr. Opin. Mol. Ther.* 1:226; Snyder et al., 2001 *Curr. Opin. Mol. Ther.* 3:147; Gariepy et al., 2001 *Trends Biotechnol.* 19:21).

Alternatively, established methodologies for introducing into a cell a DEP-1 polypeptide that is not a targeted fusion protein may be employed. For example, Chariot™ is a transfection method that quickly and efficiently delivers biologically active proteins, peptides, and antibodies directly into cultured mammalian cells. The Chariot™ peptide (available from Active Motif, Carlsbad, CA) forms a non-covalent bond with the macromolecule of interest, which stabilizes the protein, protecting it from degradation, and preserving its natural characteristics during the transfection process (Morris et al. *J. Biol. Chem.* 274 (35):24941-46 (1999); Morris, M. et al. *Nature Biotech*, 19: 1173-76 (2001)). After delivery, the complex dissociates, leaving the macromolecule biologically active and free to proceed to its target organelle. As another example, Photochemical Internalization (PCI) may be employed for

delivery of macromolecules into the cytoplasm, including proteins (*e.g.*, Selbo et al., *Int. J. Cancer* 87:853-59 (2000); Selbo et al., *Tumour Biol.* 23:103-12 (2002). Protein transduction technology has also been reviewed recently (Wadia & Dowdy, 2002 *Curr Opin Biotechnol.* 13(1):52-56) and its applicability to introducing a DEP-1 polypeptide into a cell is contemplated by the present invention.

The polypeptides of the present invention include PTP polypeptides and fusion proteins having amino acid sequences that are identical or similar to PTP sequences known in the art. For example by way of illustration and not limitation, the human PTP polypeptides (including substrate trapping mutant PTPs) referred to below in the Examples are contemplated for use according to the instant invention, as are polypeptides having at least 70% similarity (preferably 70% identity), more preferably 80% similarity (more preferably 80% identity), more preferably 90% similarity (more preferably 90% identity), more preferably 95% similarity (still more preferably 95% identity), and still more preferably 98% similarity (still more preferably 98% identity) to the polypeptides described in references cited herein and in the Examples and to portions of such polypeptides, wherein such portions of a PTP polypeptide generally contain at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and conserved amino acid substitutes thereto of the polypeptide to the sequence of a second polypeptide (*e.g.*, using GENEWORKS, Align or the BLAST algorithm, as described above). Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the nucleic acids of the present invention may be used to synthesize full-length nucleic acids of the present invention.

The term "isolated" means that the material is removed from its original environment (*e.g.*, the natural environment if it is naturally occurring). For example, a naturally occurring nucleic acid or polypeptide present in a living animal is not isolated, but the same nucleic acid or polypeptide, separated from some or all of the co-existing materials in

the natural system, is isolated. Such nucleic acid could be part of a vector and/or such nucleic acid or polypeptide could be part of a composition, and still be isolated in that such vector or composition is not part of the natural environment for the nucleic acid or polypeptide. The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region "leader and trailer" as well as intervening sequences (introns) between individual coding segments (exons).

As described herein, certain embodiments of the invention contemplate a fusion protein comprising a polypeptide of interest that is fused to a DEP-1 polypeptide, which fusion protein is encoded by nucleic acids that have the DEP-1 polypeptide coding sequence fused in frame to an additional coding sequence. The presence of such a fusion domain joined to the DEP-1 polypeptide may permit, for example by way of illustration and not limitation, detection, isolation and/or purification of the DEP-1 fusion protein by protein-protein affinity, metal affinity or charge affinity-based polypeptide purification, or by specific protease cleavage of a fusion protein containing a fusion sequence that is cleavable by a protease such that the DEP-1 polypeptide is separable from the fusion protein.

Thus, DEP-1 polypeptides may include PTP fusion proteins that comprise affinity tag polypeptide sequences, which refers to polypeptides or peptides added to DEP-1 to facilitate detection and isolation of the PTP via a specific affinity interaction with a ligand. The ligand may be any molecule, receptor, counterreceptor, antibody or the like with which the affinity tag may interact through a specific binding interaction as provided herein. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Patent No. 5,011,912 and in Hopp et al., (1988 *Bio/Technology 6*:1204), or the XPRESS™ epitope tag (Invitrogen, Carlsbad, CA). The affinity sequence may be a hexa-histidine tag as supplied, for example, by a pBAD/His (Invitrogen) or a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, for example, the affinity sequence may be a hemagglutinin (HA) tag when a mammalian host, *e.g.*, COS-7 cells, is used. The HA tag corresponds to an antibody defined epitope derived from the influenza hemagglutinin protein (Wilson et al., 1984 *Cell 37*:767).

PTP fusion proteins may further comprise immunoglobulin constant region polypeptides added to PTP to facilitate detection, isolation and/or localization of PTP. The immunoglobulin constant region polypeptide preferably is fused to the C-terminus of a PTP polypeptide. General preparation of fusion proteins comprising heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, *e.g.*, by Ashkenazi et al. (*Proc. Natl. Acad. Sci. USA 88:*10535, 1991) and Byrn et al. (*Nature 344*:677, 1990). A gene fusion encoding the PTP:Fc fusion protein is inserted into an appropriate expression vector. In certain embodiments of the invention, PTP:Fc fusion proteins may be allowed to assemble much like antibody molecules, whereupon interchain disulfide bonds form between Fc polypeptides, yielding dimeric PTP fusion proteins.

PTP fusion proteins having specific binding affinities for pre-selected antigens by virtue of fusion polypeptides comprising immunoglobulin V-region domains encoded by DNA sequences linked in-frame to sequences encoding PTP are also within the scope of the invention, including variants and fragments thereof as provided herein. General strategies for the construction of fusion proteins having immunoglobulin V-region fusion polypeptides are disclosed, for example, in EP 0318554; U.S. 5,132,405; U.S. 5,091,513; and U.S. 5,476,786.

The expressed recombinant DEP-1 polypeptides or fusion proteins (including substrate trapping mutant DEP-1) may be useful in intact host cells; in intact organelles such as cell membranes, intracellular vesicles or other cellular organelles; or in disrupted cell preparations including but not limited to cell homogenates or lysates, microsomes, uni- and multilamellar membrane vesicles or other preparations. Alternatively, expressed recombinant DEP-1 polypeptides or fusion proteins can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The nucleic acid of the present invention may also encode a fusion protein comprising a PTP polypeptide fused to other polypeptides having desirable affinity properties, for example an enzyme such as glutathione-S-transferase. As another example, PTP fusion proteins may also comprise a PTP polypeptide fused to a *Staphylococcus aureus* protein A polypeptide; protein A encoding nucleic acids and their use in constructing fusion proteins having affinity for immunoglobulin constant regions are disclosed generally, for example, in U.S. Patent 5,100,788. Other useful affinity polypeptides for construction of PTP fusion proteins may include streptavidin fusion proteins, as disclosed, for example, in WO 89/03422; U.S. 5,489,528; U.S. 5,672,691; WO 93/24631; U.S. 5,168,049; U.S. 5,272,254 and elsewhere, and avidin fusion proteins (see, *e.g.,* EP 511,747). As provided herein and in the cited references, PTP polypeptide sequences, including substrate trapping mutant PTPs, may be fused to fusion polypeptide sequences that may be full length fusion polypeptides and that may alternatively be variants or fragments thereof.

The present invention also contemplates PTP fusion proteins that contain polypeptide sequences that direct the fusion protein to the cell nucleus, to reside in the lumen of the endoplasmic reticulum (ER), to be secreted from a cell via the classical ER-Golgi secretory pathway (*see, e.g.,* von Heijne, *J. Membrane Biol. 115*:195-201, 1990), to be incorporated into the plasma membrane, to associate with a specific cytoplasmic component including the cytoplasmic domain of a transmembrane cell surface receptor or to be directed to a particular subcellular location by any of a variety of known intracellular protein sorting mechanisms with which those skilled in the art will be familiar (*See, e.g.,* Rothman, *Nature* 372:55-63, 1994, Adrani et al., 1998 *J. Biol. Chem.* 273:10317, and references cited therein.). Accordingly, these and related embodiments are encompassed by the instant compositions and methods directed to targeting a polypeptide of interest to a predefined intracellular, membrane or extracellular localization.

The present invention also relates to vectors and to constructs that include nucleic acids of the present invention, and in particular to "recombinant expression constructs" that include any nucleic acids encoding DEP-1 polypeptides according to the invention as provided above; to host cells which are genetically engineered with vectors and/or constructs

of the invention and to the production of DEP-1 polypeptides and fusion proteins of the invention, or fragments or variants thereof, by recombinant techniques. DEP-1 polypeptides can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described, for example, by Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Third Edition, Cold Spring Harbor, New York, (2001).

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, *e.g.*, the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, *e.g.*, stabilization or simplified purification of expressed recombinant product.

Useful expression constructs for bacterial use are constructed by inserting into an expression vector a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The construct may comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector construct and, if desirable, to provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice. Any other plasmid or vector may be used as long as they are replicable and viable in the host.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotech, Madison, Wisconsin, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter, if it is a regulated promoter as provided herein, is induced by appropriate means (*e.g.*, temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents; such methods are well know to those skilled in the art.

Thus, for example, the nucleic acids of the invention as provided herein may be included in any one of a variety of expression vector constructs as a recombinant expression construct for expressing a DEP-1 polypeptide. Such vectors and constructs include chromosomal, nonchromosomal and synthetic DNA sequences, *e.g.*, derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA, such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used for preparation of a recombinant expression construct as long as it is replicable and viable in the host.

The appropriate DNA sequence(s) may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques

are described, for example, in Ausubel et al. (1993 *Current Protocols in Molecular Biology*, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., Boston, MA); Sambrook et al. (2001 *Molecular Cloning*, Third Ed., Cold Spring Harbor Laboratory, Plainview, NY); Maniatis et al. (1982 Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, NY); and elsewhere.

The DNA sequence in the expression vector is operatively linked to at least one appropriate expression control sequences (*e.g.*, a promoter or a regulated promoter) to direct mRNA synthesis. Representative examples of such expression control sequences include LTR or SV40 promoter, the *E. coli lac* or *trp*, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art, and preparation of certain particularly preferred recombinant expression constructs comprising at least one promoter or regulated promoter operably linked to a nucleic acid encoding a DEP-1 polypeptide is described herein.

As noted above, in certain embodiments the vector may be a viral vector such as a retroviral vector. For example, retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The viral vector includes one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, et al., *Biotechniques* 7:980-990 (1989), or any other promoter (*e.g.*, cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β-actin promoters). Other viral

promoters that may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein, and may be from among either regulated promoters or promoters as described above.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, ψ-2, ψ-AM, PA12, T19-14X, VT-19-17-H2, ψCRE, ψCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, *Human Gene Therapy*, *1*:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and calcium phosphate precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles that include the nucleic acid sequence(s) encoding the DEP-1 polypeptides or fusion proteins. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the DEP-1 polypeptide or fusion protein. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, bronchial epithelial cells and various other culture-adapted cell lines.

As another example of an embodiment of the invention in which a viral vector is used to prepare the recombinant DEP-1 expression construct, in one preferred embodiment, host cells transduced by a recombinant viral construct directing the expression of DEP-1 polypeptides or fusion proteins may produce viral particles containing expressed PTP polypeptides or fusion proteins that are derived from portions of a host cell membrane incorporated by the viral particles during viral budding. In another preferred embodiment, PTP encoding nucleic acid sequences are cloned into a baculovirus shuttle vector, which is then recombined with a baculovirus to generate a recombinant baculovirus expression

construct that is used to infect, for example, Sf9 host cells, as described in *Baculovirus Expression Protocols, Methods in Molecular Biology* Vol. 39, Christopher D. Richardson, Editor, Human Press, Totowa, NJ, 1995; Piwnica-Worms, "Expression of Proteins in Insect Cells Using Baculoviral Vectors," Section II in Chapter 16 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel *et al.*, eds., John Wiley & Sons, New York, New York, 1992, pages 16-32 to 16-48.

In another aspect, the present invention relates to host cells containing the above described recombinant DEP-1 expression constructs. Host cells are genetically engineered (transduced, transformed, or transfected) with the vectors and/or expression constructs of this invention that may be, for example, a cloning vector, a shuttle vector or an expression construct. The vector or construct may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying particular genes such as genes encoding DEP-1 polypeptides or DEP-1 fusion proteins. The culture conditions for particular host cells selected for expression, such as temperature, pH and the like, will be readily apparent to the ordinarily skilled artisan.

The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Representative examples of appropriate host cells according to the present invention include, but need not be limited to, bacterial cells, such as *E. coli, Streptomyces, Salmonella typhimurium*; fungal cells, such as yeast; insect cells, such as *Drosophila S2* and *Spodoptera Sf9*; animal cells, such as CHO, COS or 293 cells; plant cells, or any suitable cell already adapted to *in vitro* propagation or so established *de novo*. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

Various mammalian cell culture systems can also be employed to express recombinant protein. The invention is therefore directed in part to a method of producing a recombinant DEP-1 polypeptide, by culturing a host cell comprising a recombinant expression construct that comprises at least one promoter operably linked to a nucleic acid sequence

encoding the DEP-1 polypeptide, wherein the promoter may be a regulated promoter as provided herein, for example a tetracylcine-repressible promoter. In certain embodiments the recombinant expression construct is a recombinant viral expression construct as provided herein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell 23*:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5′ flanking nontranscribed sequences, for example as described herein regarding the preparation of PTP expression constructs. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Introduction of the construct into the host cell can be effected by a variety of methods with which those skilled in the art will be familiar, including but not limited to, for example, calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis et al., 1986 *Basic Methods in Molecular Biology*).

In certain particularly preferred embodiments, the present invention provides host cells capable of expressing a DEP-1 polypeptide following a growth period for cell propagation. By way of background, attempts to express DEP-1 constitutively in breast cells and macrophages (Keane et al., *supra*; Osborne et al., *supra*) have apparently been hindered by DEP-1-mediated growth inhibition, precluding development of stable cell lines. In order to overcome this limitation, according to the present invention a recombinant expression construct is provided that comprises a regulated promoter that is operably linked to a polynucleotide encoding a DEP-1 polypeptide. Preferably the regulated promoter is an inducible promoter, and still more preferably the promoter is a tightly regulated promoter. According to non-limiting theory, the use of a tightly regulated promoter that permits little or no transcription of the DEP-1-encoding polynucleotide permits growth of host cells that have stably incorporated the subject invention recombinant expression construct, such that cell growth is not impaired by the growth inhibitory effects of DEP-1 polypeptides. Further

according to theory, only at a desired time, for instance after a population of host cells has been grown to a useful quantity, can DEP-1 expression be induced by contacting the cells with an appropriate inducing agent that activates the inducible promoter or the tightly regulated promoter. Such host cells may then be employed in the methods of the present invention, such as screening methods for agents that alter DEP-1 interaction with substrates, or that alter DEP-1 dephosphorylation of substrates. Preferably the host cell can be adapted to sustained propagation in culture to yield a cell line according to art-established methodologies. In certain preferred embodiments the cell line is an immortal cell line, which refers to a cell line that can be repeatedly (and at least ten times while remaining viable) passaged in culture following log-phase growth. In other preferred embodiments the host cell used to generate a cell line according to the invention is a cell that is capable of unregulated growth, such as a cancer cell, or a transformed cell, or a malignant cell.

Design and selection of inducible, regulated promoters and/or tightly regulated promoters are known in the art and will depend on the particular host cell and expression system. The pBAD Expression System (Invitrogen Life Technologies, Carlsbad, CA) is an example of a tightly regulated expression system that uses the *E. coli* arabinose operon (P_{BAD} or P_{ARA}) (see Guzman et al., *J. Bacteriology* 177:4121-30 (1995); Smith et al., *J. Biol. Chem.* 253:6931-33 (1978); Hirsh et al., *Cell* 11:545-50 (1977)), which controls the arabinose metabolic pathway. A variety of vectors employing this system are commercially available. Other examples of tightly regulated promoter-driven expression systems include PET Expression Systems (U.S. Pat. No. 4.952,496) available from Stratagene (La Jolla, CA) or tet-regulated expression systems (Gossen & Bujard, *Proc. Natl. Acad. Sci. USA* 89:5547-51 (1992) and Gossen et al., *Science* 268:1766-69 (1995)). The pLP-TRE2 Acceptor Vector (BD Biosciences Clontech, Palo Alto, CA) is designed for use with CLONTECH's Creator™ Cloning Kits to rapidly generate a tetracycline-regulated expression construct for tightly controlled, inducible expression of a gene of interest using the site-specific Cre-lox recombination system (*e.g.*, Sauer, 1998 *Methods* 14:381; Furth, 1997 *J. Mamm. Gland Biol. Neoplas.* 2:373), which may also be employed for host cell immortalization (*e.g.*, Cascio, 2001 *Artif. Organs* 25:529).

Identification of nucleic acid molecules for use as antisense agents, which includes antisense oligonucleotides and ribozymes specific for nucleic acid sequences encoding DEP-1 (including substrate trapping mutant DEP-1) or variants or fragments thereof; and of DNA oligonucleotides encoding DEP-1 genes (including substrate trapping mutant DEP-1) for targeted delivery for genetic therapy, involve methods well known in the art. For example, the desirable properties, lengths and other characteristics of such oligonucleotides are well known. In certain preferred embodiments such an antisense oligonucleotide comprises at least 15 consecutive nucleotides complementary to an isolated nucleic acid molecule encoding a substrate trapping mutant PTP as provided herein. Antisense oligonucleotides are typically designed to resist degradation by endogenous nucleolytic enzymes by using such linkages as: phosphorothioate, methylphosphonate, sulfone, sulfate, ketyl, phosphorodithioate, phosphoramidate, phosphate esters, and other such linkages (*see*, *e.g.*, Agrwal et al., *Tetrehedron Lett. 28*:3539-3542 (1987); Miller et al., *J. Am. Chem. Soc. 93*:6657-6665 (1971); Stec et al., *Tetrehedron Lett. 26*:2191-2194 (1985); Moody et al., *Nucleic Acids Res. 12*:4769-4782 (1989); Uznanski et al., *Nucleic Acids Res.* (1989); Letsinger et al., *Tetrahedron 40*:137-143 (1984); Eckstein, *Annu. Rev. Biochem. 54*:367-402 (1985); Eckstein, *Trends Biol. Sci. 14*:97-100 (1989); Stein In: *Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression*, Cohen, Ed, Macmillan Press, London, pp. 97-117 (1989); Jager et al., *Biochemistry 27*:7237-7246 (1988)).

Antisense nucleotides are oligonucleotides that bind in a sequence-specific manner to nucleic acids, such as mRNA or DNA. When bound to mRNA that has complementary sequences, antisense prevents translation of the mRNA (*see*, *e.g.*, U.S. Patent No. 5,168,053 to Altman et al.; U.S. Patent No. 5,190,931 to Inouye, U.S. Patent No. 5,135,917 to Burch; U.S. Patent No. 5,087,617 to Smith and Clusel et al. (1993) *Nucleic Acids Res. 21*:3405-3411; which describes dumbbell antisense oligonucleotides). Triplex molecules refer to single DNA strands that bind duplex DNA forming a colinear triplex molecule, thereby preventing transcription (*see*, *e.g.*, U.S. Patent No. 5,176,996 to Hogan et al., which describes methods for making synthetic oligonucleotides that bind to target sites on duplex DNA).

According to this embodiment of the invention, particularly useful antisense nucleotides and triplex molecules are molecules that are complementary to or bind the sense strand of DNA or mRNA that encodes a PTP polypeptide (including substrate trapping mutant DEP-1), such that inhibition of translation of mRNA encoding the DEP-1 polypeptide is effected.

A ribozyme is an RNA molecule that specifically cleaves RNA substrates, such as mRNA, resulting in specific inhibition or interference with cellular gene expression. There are at least five known classes of ribozymes involved in the cleavage and/or ligation of RNA chains.. Ribozymes can be targeted to any RNA transcript and can catalytically cleave such transcripts (*see, e.g.*, U.S. Patent No. 5,272,262; U.S. Patent No. 5,144,019; and U.S. Patent Nos. 5,168,053, 5,180,818, 5,116,742 and 5,093,246 to Cech et al.). According to certain embodiments of the invention, any such PTP (including substrate trapping mutant PTP) mRNA-specific ribozyme, or a nucleic acid encoding such a ribozyme, may be delivered to a host cell to effect inhibition of PTP gene expression. Ribozymes, and the like may therefore be delivered to the host cells by DNA encoding the ribozyme linked to a eukaryotic promoter, such as a eukaryotic viral promoter, such that upon introduction into the nucleus, the ribozyme will be directly transcribed.

A biological signaling pathway may be induced in subject or biological source cells by contacting such cells with an appropriate stimulus, which may vary depending upon the signaling pathway under investigation, whether known or unknown. For example, a signaling pathway that, when induced, results in protein tyrosine phosphorylation and/or protein tyrosine dephosphorylation may be stimulated in subject or biological source cells using any one or more of a variety of well known methods and compositions known in the art to stimulate protein tyrosine kinase and/or PTP (*e.g.*, DEP-1) activity. These stimuli may include, without limitation, exposure of cells to cytokines, growth factors, hormones, peptides, small molecule mediators, cell stressors (*e.g.*, ultraviolet light; temperature shifts; osmotic shock; ROS or a source thereof, such as hydrogen peroxide, superoxide, ozone, etc. or any agent that induces or promotes ROS production (*see, e.g.*, Halliwell and Gutteridge, *Free Radicals in Biology and Medicine* (3rd Ed.) 1999 Oxford University Press, Oxford, UK); heavy

metals; alcohol) or other agents that induce PTK-mediated protein tyrosine phosphorylation and/or PTP-mediated phosphoprotein tyrosine dephosphorylation. Such agents may include, for example, interleukins (*e.g.*, IL-1, IL-3), interferons (*e.g.*, IFN-γ), human growth hormone, insulin, epidermal growth factor (EGF), platelet derived growth factor (PDGF), granulocyte colony stimulating factor (G-CSF), granulocyte-megakaryocyte colony stimulating factor (GM-CSF), transforming growth factor (*e.g.*, TGF-β1), tumor necrosis factor (*e.g.*, TNF-α) and fibroblast growth factor (FGF; *e.g.*, basic FGF (bFGF)), any agent or combination of agents capable of triggering T lymphocyte activation via the T cell receptor for antigen (TCR; TCR-inducing agents may include superantigens, specifically recognized antigens and/or MHC-derived peptides, MHC peptide tetramers (*e.g.*, Altman et al., 1996 *Science* 274:94-96) TCR-specific antibodies or fragments or derivatives thereof), lectins (*e.g.*, PHA, PWM, ConA, etc.), mitogens, G-protein coupled receptor agonists such as angiotensin-2, thrombin, thyrotropin, parathyroid hormone, lysophosphatidic acid (LPA), sphingosine-1-phosphate, serotonin, endothelin, acetylcholine, platelet activating factor (PAF) or bradykinin, as well as other agents with which those having ordinary skill in the art will be familiar (*see, e.g.*, Rhee et al., Sci STKE. 2000 Oct 10;2000(53):PE1 and references cited therein).

As noted above, regulated tyrosine phosphorylation contributes to specific pathways for biological signal transduction, including those associated with cell division, cell survival, apoptosis, proliferation and differentiation, and "inducible signaling pathways" in the context of the present invention include transient or stable associations or interactions among molecular components involved in the control of these and similar processes in cells. Depending on the particular pathway of interest, an appropriate parameter for determining induction of such pathway may be selected. For example, for signaling pathways associated with cell proliferation, there is available a variety of well known methodologies for quantifying proliferation, including, for example, incorporation of tritiated thymidine into cellular DNA, monitoring of detectable (*e.g.*, fluorimetric or colorimetric) indicators of cellular respiratory activity, or cell counting, or the like. Similarly, in the cell biology arts there are known multiple techniques for assessing cell survival (*e.g.*, vital dyes, metabolic indicators, etc.) and for determining apoptosis (*e.g.*, annexin V binding, DNA fragmentation assays,

caspase activation, etc.). Other signaling pathways will be associated with particular cellular phenotypes, for example specific induction of gene expression (*e.g.*, detectable as transcription or translation products, or by bioassays of such products, or as nuclear localization of cytoplasmic factors), altered (*e.g.*, statistically significant increases or decreases) levels of intracellular mediators (*e.g.*, activated kinases or phosphatases, altered levels of cyclic nucleotides or of physiologically active ionic species, etc.), or altered cellular morphology, and the like, such that cellular responsiveness to a particular stimulus as provided herein can be readily identified to determine whether a particular cell comprises an inducible signaling pathway. For example, given the disclosure provided herein for the first time that DEP-1 associates with, and is capable of being isolated in a complex with, the Met cell surface receptor, certain cellular morphogenetic and motility properties associated with Met activity may provide evidence of biological signal transduction in a cell (*e.g.*, Vadnais et al., 2002 *J. Biol. Chem.* [epub ahead of print], Manuscript M209481200, October 7, 2002).

A "sample" as used herein refers to a biological sample containing at least one tyrosine phosphorylated protein, and may be provided by obtaining a blood sample, biopsy specimen, tissue explant, organ culture or any other tissue or cell preparation from a subject or a biological source. A sample may further refer to a tissue or cell preparation in which the morphological integrity or physical state has been disrupted, for example, by dissection, dissociation, solubilization, fractionation, homogenization, biochemical or chemical extraction, pulverization, lyophilization, sonication or any other means for processing a sample derived from a subject or biological source. In certain preferred embodiments, the sample is a cell lysate, and in certain particularly preferred embodiments the lysate is a detergent solubilized cell lysate from which insoluble components have been removed according to standard cell biology techniques. The subject or biological source may be a human or non-human animal, a primary cell culture or culture adapted cell line including but not limited to genetically engineered cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, immortalized or immortalizable cell lines, somatic cell hybrid cell lines, differentiated or differentiatable cell lines, transformed cell lines and the like. Optionally, in certain situations it may be desirable to treat cells in a biological sample with

pervanadate as described herein, to enrich the sample in tyrosine phosphorylated proteins. Other means may also be employed to effect an increase in the population of tyrosine phosphorylated proteins present in the sample, including the use of a subject or biological source that is a cell line that has been transfected with at least one gene encoding a protein tyrosine kinases. Additionally or alternatively, protein tyrosine phosphorylation may be stimulated in subject or biological source cells using any one or more of a variety of well known methods and compositions known in the art to stimulate protein tyrosine kinase activity. These stimuli may include, without limitation, exposure of cells to cytokines, growth factors, hormones, peptides, small molecule mediators or other agents that induce PTK-mediated protein tyrosine phosphorylation. Such agents may include, for example, interleukins, interferons, human growth hormone, insulin and fibroblast growth factor (FGF), as well as other agents with which those having ordinary skill in the art will be familiar.

According to the subject invention, a sample comprising at least one tyrosine phosphorylated protein or polypeptide is combined with at least one substrate trapping mutant PTP as provided herein, under conditions and for a time sufficient to permit formation of a complex between the tyrosine phosphorylated protein and the substrate trapping mutant PTP. Suitable conditions for formation of such complexes are known in the art and can be readily determined based on teachings provided herein, including solution conditions and methods for detecting the presence of a complex. Next, the presence or absence of a complex comprising the tyrosine phosphorylated protein and the substrate trapping mutant PTP is determined, wherein the presence of the complex indicates that the tyrosine phosphorylated protein is a substrate of the PTP with which it forms a complex.

Substrate trapping mutant PTPs that associate in complexes with tyrosine phosphorylated protein substrates may be identified by any of a variety of techniques known in the art for demonstrating an intermolecular interaction between a PTP and a PTP substrate as described above, for example, co-purification, co-precipitation, co-immunoprecipitation, radiometric or fluorimetric assays, western immunoblot analyses, affinity capture including affinity techniques such as solid-phase ligand-counterligand sorbent techniques, affinity chromatography and surface affinity plasmon resonance, and the like (see, *e.g.,* U.S. Patent

No. 5,352,660). Determination of the presence of a PTP/substrate complex may employ antibodies, including monoclonal, polyclonal, chimeric and single-chain antibodies, and the like, that specifically bind to the PTP or the tyrosine phosphorylated protein substrate. Labeled PTPs and/or labeled tyrosine phosphorylated substrates can also be used to detect the presence of a complex. The PTP or phosphorylated protein can be labeled by covalently or non-covalently attaching a suitable reporter molecule or moiety, for example any of various enzymes, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include, but are not limited to, horseradish peroxidase, biotin, alkaline phosphatase, β-galactosidase and acetylcholinesterase. Examples of suitable fluorescent materials include, but are not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin. Appropriate luminescent materials include luminol, and suitable radioactive materials include radioactive phosphorus [^{32}P], iodine [^{125}I or ^{131}I] or tritium [^{3}H].

Using such approaches, representative complexes of PTP1B with p210 bcr:abl, of PTP-PEST with p130^{cas}, of TC-PTP with Shc (*e.g.,* Tiganis et al., 1998 *Mol. Cell. Biol.* 18:1622-1634) and of PTPH1 with pp97/VCP may be readily identified by western immunoblot analysis as described below. These associations may be observed, for example, in lysates from several cell lines and in transfected cells, indicating that p210 bcr:abl, p130^{cas}, Shc and VCP represent major physiologically relevant substrates for PTP1B, PTP-PEST, TC-PTP and PTPH1, respectively. The compositions and methods of the present invention, which may be used, as exemplified herein, to identify specific tyrosine phosphorylated substrates for PTP1B, PTP-PEST and PTPH1, are generally applicable to any member of the PTP family, including but not limited to TC-PTP, PTPγ, MKP-1, DEP-1, PTPμ, SHP2, PTP-PEZ, PTP-MEG1, LC-PTP, CD45, LAR and PTPX10.

In certain embodiments of this aspect of the invention, the sample may comprise a cell that naturally expresses the tyrosine phosphorylated protein that is a PTP substrate, while in certain other embodiments the sample may comprise a cell that has been transfected with one or more nucleic acid molecules encoding the substrate protein. For example, the sample may comprise a cell or population of cells that has been transfected with a

nucleic acid library such as a cDNA library that contains at least one nucleic acid molecule encoding a substrate protein. Any tyrosine phosphorylated protein is suitable as a potential substrate in the present invention. Tyrosine phosphorylated proteins are well known in the art. Specific examples of appropriate substrates include, without limitation, $p130^{cas}$, pp97/VCP, the EGF receptor, p210 bcr:abl, MAP kinase, Shc and the insulin receptor. Of particular interest are tyrosine phosphorylated proteins that have been implicated in a mammalian disease or disorder.

According to the present invention, substrates may include full length tyrosine phosphorylated proteins and polypeptides as well as fragments (*e.g.*, portions), derivatives or analogs thereof that can be phosphorylated at a tyrosine residue. Such fragments, derivatives and analogs include any PTP substrate polypeptide that retains at least the biological function of interacting with a PTP as provided herein, for example by forming a complex with a PTP. A fragment, derivative or analog of a PTP substrate polypeptide, including substrates that are fusion proteins, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue), and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the substrate polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (*e.g.*, polyethylene glycol) or a detectable moiety such as a reporter molecule, or (iv) one in which additional amino acids are fused to the substrate polypeptide, including amino acids that are employed for purification of the substrate polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art.

The subject invention also contemplates certain embodiments wherein the substrate trapping mutant PTP (that is combined with the sample) is a mutant PTP that is expressed by a cell, including embodiments wherein the cell has been transfected with one or more nucleic acid molecules encoding the mutant PTP. Thus, the method of identifying a tyrosine phosphorylated protein which is a substrate of a PTP may include in certain embodiments combining a sample comprising a tyrosine phosphorylated protein with a mutant

PTP wherein the sample comprises a cell expressing either or both of the tyrosine phosphorylated protein and the mutant PTP. Optionally, the cell may be transfected with nucleic acids encoding either or both of the tyrosine phosphorylated protein and the mutant PTP.

In another aspect, the invention provides methods of identifying an agent that alters the interaction between a PTP and a tyrosine phosphorylated protein that is a substrate of the PTP, through the use of screening assays that detect the ability of a candidate agent to alter (*i.e.,* increase or decrease) such interaction. The interaction between the PTP and its substrate may be determined enzymatically, for example by detecting catalytic substrate dephosphorylation. Alternatively, the interaction between the PTP (including a substrate trapping mutant PTP) and its substrate may be determined as a binding interaction, and in preferred embodiments such interaction is manifested as detection of a complex formed by PTP-substrate binding, according to criteria described herein. Agents identified according to these methods may be agonists (*e.g.,* agents that enhance or increase the activity of the wildtype PTP) or antagonists (*e.g.,* agents that inhibit or decrease the activity of the wildtype PTP) of PTP activity. Agents may be identified from among naturally occurring or non-naturally occurring compounds, including synthetic small molecules as described below.

In certain embodiments, wherein the screening assay is directed to PTP catalytic activity, the tyrosine phosphorylated protein that is a substrate of the PTP can be identified as described above, which method features the use of a novel substrate trapping mutant PTP as disclosed herein. Accordingly, a PTP and a tyrosine phosphorylated substrate are combined in the absence and in the presence of a candidate agent, where the substrate has first been identified as described above using a substrate trapping mutant PTP. The PTP and the substrate are combined under conditions permissive for the detectable dephosphorylation of the substrate to occur.

Any suitable method may be used to detect phosphoprotein dephosphorylation; such methods are well known in the art and include, without limitation, detection of substrate catalysis by one or more of, *e.g.,* radiometric, fluorimetric, densitometric, spectrophotometric, chromatographic, electrophoretic, colorimetric or biometric assays. The level of

dephosphorylation of the substrate in the absence of the agent is compared to the level of dephosphorylation of the substrate in the presence of the agent, such that a difference in the level of substrate dephosphorylation (*e.g.*, a statistically significant increase or decrease) indicates the agent alters the interaction between the protein tyrosine phosphatase and the substrate.

For instance, an enzymatic activity assay utilizing a wildtype PTP can be carried out in the absence and presence of a candidate agent. Enzymatic activity assays known in the art include, for example, PTP activity assays using a tyrosine phosphorylated ^{32}P-labeled substrate as described in Flint et al. (1993 *EMBO J. 12*:1937-1946). A decrease in the PTP enzymatic activity in the presence of the candidate agent indicates that the agent inhibits the interaction between the PTP and its substrate. Conversely, an increase in PTP enzymatic activity in the presence of the agent indicates that the agent enhances the interaction between the PTP and its substrate.

In certain other embodiments, wherein the screening assay is directed to identifying an agent capable of altering a substrate trapping mutant PTP:substrate binding interaction, the substrate trapping mutant PTP (as described herein) and a tyrosine phosphorylated substrate are combined in the absence and in the presence of a candidate agent under conditions and for a time sufficient to permit formation of a complex between the tyrosine phosphorylated substrate protein and the substrate trapping mutant PTP, thereby producing a combination. The formation of a complex comprising the tyrosine phosphorylated substrate protein and the substrate trapping mutant protein tyrosine phosphatase in the combination is next determined (as also provided herein), wherein a difference between the level of complex formation (*e.g.*, a statistically significant difference) in the absence and in the presence of the agent indicates that the agent alters (*i.e.*, increases or decreases) the interaction between the protein tyrosine phosphatase and the substrate. Alternatively, a competitive binding assay can be carried out utilizing the substrate trapping mutant PTP in the absence and presence of a candidate agent. Competitive binding assays known in the art include, for example, U.S. Patent No. 5,352,660, which describes methods suitable for use according to these embodiments of the present invention. A decrease in the extent of PTP-substrate binding

in the presence of the agent to be tested indicates that the agent inhibits the interaction between the PTP and its substrate. Conversely, an increase in the extent of binding in the presence of the agent to be tested indicates that the agent enhances the interaction between the PTP and its substrate.

Candidate agents for use in a method of screening for an agent that alters the interaction between a PTP and its tyrosine phosphorylated protein substrate according to the present invention (*e.g.*, an inhibitor of PTP1B binding to a PTP1B substrate) may be provided as "libraries" or collections of compounds, compositions or molecules. Candidate agents that may interact with one or more PTPs (including agents that interact with a substrate trapping mutant PTP as provided herein) may include members of phosphotyrosyl peptide libraries as described in Songyang et al. (1995 *Nature 373*:536-539; 1993 *Cell 72*:767-778) that bind to the PTP. Peptides identified from such peptide libraries can then be assessed to determine whether tyrosine phosphorylated proteins containing these peptides exist in nature. Alternatively, libraries of candidate molecules to be screened may typically include compounds known in the art as "small molecules" and having molecular weights less than 10^5 daltons, preferably less than 10^4 daltons and still more preferably less than 10^3 daltons. For example, members of a library of test compounds can be administered to a plurality of samples, each containing at least one substrate trapping mutant PTP and at least one tyrosine phosphorylated protein that is a substrate of the PTP as provided herein, and then assayed for their ability to enhance or inhibit mutant PTP binding to the substrate. Compounds so identified as capable of altering PTP-substrate interaction (*e.g.*, binding and/or substrate phosphotyrosine dephosphorylation) are valuable for therapeutic and/or diagnostic purposes, since they permit treatment and/or detection of diseases associated with PTP activity. Such compounds are also valuable in research directed to molecular signaling mechanisms that involve PTPs, and to refinements in the discovery and development of future compounds exhibiting greater specificity.

Candidate agents further may be provided as members of a combinatorial library, which preferably includes synthetic agents prepared according to a plurality of predetermined chemical reactions performed in a plurality of reaction vessels. For example,

various starting compounds may be prepared employing one or more of solid-phase synthesis, recorded random mix methodologies and recorded reaction split techniques that permit a given constituent to traceably undergo a plurality of permutations and/or combinations of reaction conditions. The resulting products comprise a library that can be screened followed by iterative selection and synthesis procedures, such as a synthetic combinatorial library of peptides (see *e.g.,* PCT/US91/08694, PCT/US91/04666, which are hereby incorporated by reference in their entireties) or other compositions that may include small molecules as provided herein (see *e.g.,* PCT/US94/08542, EP 0774464, U.S. 5,798,035, U.S. 5,789,172, U.S. 5,751,629, which are hereby incorporated by reference in their entireties). Those having ordinary skill in the art will appreciate that a diverse assortment of such libraries may be prepared according to established procedures, and tested using substrate trapping mutant PTPs according to the present disclosure.

Similarly, the invention relates to a method of reducing the formation of inducible or induced signaling complexes associated with PTP-mediated pathways, and in preferred embodiments DEP-1-mediated biological signaling pathways as known to the art and as disclosed herein. DEP-1 overexpression in a cell comprising an inducible biological signaling pathway, which cell has been contacted with a stimulus that induces the pathway to generate an increased level of a molecular complex comprising DEP-1 and a DEP-1 substrate polypeptide as provided herein, may also be used to alter (*i.e.,* increase or decrease) a DEP-1-mediated biological signal with therapeutic benefit.

The methods of the present invention are specifically exemplified herein with respect to the DEP-1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 and may also in certain preferred embodiments relate to the DEP-1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:3; however, it is understood that the invention is not limited to these specific DEP-1 polypeptides but may be applicable to certain other DEP-1 polypeptides as provided herein. In certain embodiments, the invention relates in part to DEP-1(D1205A), in which the aspartate residue at position 1205 of wildtype DEP-1 (SEQ ID NO:2) is replaced with alanine, and in which further a PTP tyrosine residue may optionally be replaced with a non-phosphorylatable residue.

As disclosed herein and described in the Examples, the substrate specificities of DEP-1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 may be characterized by methods that relate to PTP catalytic and/or binding interactions with substrate, *e.g.*, dephosphorylation and substrate trapping *in vitro* and *in vivo*. DEP-1 (see, *e.g.*, U.S. Pat. No. 6,114,140; WO 95/30008) is well known in the art. The substrate trapping methods provided herein are generally applicable to any DEP-1 polypeptide by virtue of the invariant PTP catalytic domain aspartate residue and the frequency of tyrosine in PTP amino acid sequences, and should therefore prove useful in delineating the substrate preferences of other PTP family members. In particular, the use of mutant, catalytically impaired PTPs to trap, and thereby isolate, potential substrates permits the identification of physiologically important substrates for individual PTPs, leading to improved understanding of the roles of these enzymes in regulation of cellular processes. Furthermore, replacement of PTP tyrosine residues with amino acids that cannot be phosphorylated provides substrate trapping mutant PTPs that are not impaired in their ability to interact with tyrosine phosphorylated protein substrates.

The present invention also pertains to pharmaceutical compositions comprising an agent that is capable of altering the specific association of a DEP-1 polypeptide with a DEP-1 substrate polypeptide. For administration to a patient, one or more such agents are generally formulated as a pharmaceutical composition. A pharmaceutical composition may be a sterile aqueous or non-aqueous solution, suspension or emulsion, which additionally comprises a physiologically acceptable carrier (*i.e.*, a non-toxic material that does not interfere with the activity of the active ingredient). Such compositions may be in the form of a solid, liquid or gas (aerosol). Alternatively, compositions of the present invention may be formulated as a lyophilizate or compounds may be encapsulated within liposomes using well known technology. Pharmaceutical compositions within the scope of the present invention may also contain other components, which may be biologically active or inactive. Such components include, but are not limited to, buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as

EDTA or glutathione, stabilizers, dyes, flavoring agents, and suspending agents and/or preservatives.

Any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of the present invention. Carriers for therapeutic use are well known, and are described, for example, in *Remingtons Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro ed. 1985). In general, the type of carrier is selected based on the mode of administration. Pharmaceutical compositions may be formulated for any appropriate manner of administration, including, for example, topical, oral, nasal, intraocular, intrathecal, rectal, vaginal, sublingual or parenteral administration, including subcutaneous, intravenous, intramuscular, intrasternal, intracavernous, intrameatal or intraurethral injection or infusion. For parenteral administration, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose, ethyl cellulose, glucose, sucrose and/or magnesium carbonate, may be employed.

A pharmaceutical composition (*e.g.*, for oral administration or delivery by injection) may be in the form of a liquid (*e.g.*, an elixir, syrup, solution, emulsion or suspension). A liquid pharmaceutical composition may include, for example, one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. The use of physiological saline is preferred, and an injectable pharmaceutical composition is preferably sterile.

The compositions described herein may be formulated for sustained release (*i.e.,* a formulation such as a capsule or sponge that effects a slow release of compound following administration). Such compositions may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain an agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

For pharmaceutical compositions comprising an agent that is a nucleic acid molecule encoding a DEP-1 polypeptide or a DEP-1 substrate polypeptide that is capable of altering the specific association of a DEP-1 polypeptide with a DEP-1 substrate polypeptide (such that the polypeptide is generated *in situ*), the nucleic acid molecule may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, and bacterial, viral and mammalian expression systems such as, for example, recombinant expression constructs as provided herein. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

Within a pharmaceutical composition, a DEP-1 or a DEP-1 substrate polypeptide, a DEP-1- or a DEP-1 substrate-encoding nucleic acid molecule or an agent that is capable of altering the specific association of a DEP-1 polypeptide with a DEP-1 substrate polypeptide may be linked to any of a variety of compounds. For example, such a polypeptide, nucleic acid molecule or agent may be linked to a targeting moiety (*e.g.,* a monoclonal or polyclonal antibody, a protein or a liposome) that facilitates the delivery of the agent to the

target site. As used herein, a "targeting moiety" may be any substance (such as a compound or cell) which, when linked to an agent, enhances the transport of the agent to a target cell or tissue, thereby increasing the local concentration of the agent. Targeting moieties include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. An antibody targeting agent may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are F(ab')2, -Fab', Fab and F[v] fragments, which may be produced by conventional methods or by genetic or protein engineering. Linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers. Targeting moieties may be selected based on the cell(s) or tissue(s) at which the agent is expected to exert a therapeutic benefit.

Pharmaceutical compositions may be administered in a manner appropriate to the disease to be treated (or prevented), for example, a condition, disorder or disease associated with cell growth, differentiation or survival, such as cancer or any other malignant condition, autoimmune disease, inflammatory disease or any other condition wherein a beneficial response may be elicited by specific manipulation of a DEP-1 signal transduction pathway. An appropriate dosage and a suitable duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease, the particular form of the active ingredient and the method of administration. In general, an appropriate dosage and treatment regimen provides the agent(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit (*e.g.*, an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free and/or overall survival). For prophylactic use, a dose should be sufficient to prevent, delay the onset of or diminish the severity of a disease associated with a defect in cell signaling, for example a defect leading to abnormal cell cycle regulation, proliferation, activation, differentiation, senescence, apoptosis, adhesion, metabolic activity, gene expression or the like.

Optimal dosages may generally be determined using experimental models and/or clinical trials. In general, the amount of polypeptide present in a dose, or produced *in situ* by DNA present in a dose, ranges from about 0.01 μg to about 100 μg per kg of host,

typically from about 0.1 μg to about 10 μg. The use of the minimum dosage that is sufficient to provide effective therapy is usually preferred. Patients may generally be monitored for therapeutic or prophylactic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those having ordinary skill in the art. Suitable dose sizes will vary with the size of the patient, but will typically range from about 1 mL to about 500 mL for a 10-60 kg subject.

All of the above U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference, in their entirety.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

EXAMPLE 1

EXPERIMENTAL PROCEDURES

Generation of DEP-1 cDNA constructs - Full-length human DEP-1 cDNA was isolated and subcloned into the mammalian expression vector pMT2 (Ostman et al., *Proc. Natl. Acad. Sci. USA* 91:9680-84 (1994)). The nucleotide and amino acid numbers listed below correspond to the human DEP-1 sequence reported previously (Ostman et al., *supra*) GenBank Accession Number U10886. DEP-1 point mutants (C1239S, D1205A) were generated by overlap extension using pMT2.DEP-1 as template. The resulting mutant PCR products were exchanged with wild type sequence in pMT2.DEP-1 and sequenced to confirm the mutations. As used herein, a polynucleotide encoding a DEP-1(DA) mutant describes a DEP-1 mutant that has the aspartate residue at position 1205 of SEQ ID NO:2 substituted with an alanine residue, and a polynucleotide encoding a DEP-1(CS) mutant describes a DEP-1 mutant that has the cysteine residue at position 1239 of SEQ ID NO:2 substituted with a serine residue.

DEP-1 cytoplasmic domain constructs were generated using the pMT2.DEP-1 wild type or point mutant (C1239S, D1205A) constructs as template. A 5' primer introduced a BamHI site before the DEP-1 cytoplasmic sequence at nucleotide 3338, whereas a 3' primer added a SalI site after the DEP-1 stop codon. The resulting PCR products (DEP-1 nucleotides 3338-4362) were cloned into the BamHI/SalI sites of the pMAL-c2E vector from New England Biolabs (Beverly, MA) generating wild type and point mutant (C1239S, D1205A) pMAL.DEP-1 constructs. The fusion proteins were expressed in *Escherichia coli* and purified on amylose resin according to the manufacturer's instructions. The resulting proteins (~84 kDa) have maltose binding protein (MBP) fused to the N-terminus of the DEP-1 cytoplasmic domain (amino acids 997-1337).

Met chimeric construct - The chimeric receptor CSF-MET comprising the extracellular domain of human CSF-1R and the transmembrane and cytoplasmic domains of

human Met was described by Zhu et al., (1994) *supra*. Briefly, the human extracellular domain of the CSF-MET fusion protein corresponded to amino acids at positions 1-507 of CSF-1R (*see, e.g.,* GenBank Acc. No. NP_005202; Acc. No. 1204266A; and Acc. No. PO7333). The histidine at position 508 in CSF (*see* Acc. No. NP_005202) was mutated to an aspartic acid residue to generate a restriction site for cloning purposes (*see* Zhu et al., (1994) *supra*, Figure 1). To this aspartate was fused the MET transmembrane and cytoplasmic domains (amino acids at positions 938-1408 of MET proto-oncogene, Zhu et al., (1994) *supra*; *see* GenBank Acc. No. NP_000236; Acc. No. AAA59591).

Cell culture and transfections - MDA-MB-231 (ATTC HTB-26) and T-47D (ATCC HTB-133) human breast tumor cells (American Type Culture Collection, Manassas, VA) were cultured in DMEM containing 5% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin and 1% non-essential amino acids. The T-47D/Met cell line (Shen et al., *Cell* 103:501-10 (2000)) was cultured in DMEM as above further supplemented with 200 μg/ml G418. Human embryonal kidney 293 cells (ATTC CRL-1573) were cultured in DMEM containing 10% bovine calf serum, 100 units/ml penicillin and 100 μg/ml streptomycin.

Transfection of 293 cells was performed using the calcium phosphate-mediated transfection protocol. For trapping experiments, 293 cells were transfected with 20 μg CSF-MET DNA (pXM.CSF-MET) and 20 μg of empty vector DNA (pMT2) or 20 μg DEP-1 DNA (pMT2.DEP-1, pMT2.DEP-1(CS), pMT2.DEP-1(DA)) per 10 cm dish. To examine dephosphorylation in 293 cells, 20 μg CSF-MET DNA (pXM.CSF-MET) were co-transfected with increasing amounts of DEP-1 DNA (pMT2.DEP-1) (0, 1, 2, 5, 10 μg) or 10 μg DEP-1(CS) DNA (pMT2.DEP-1(CS)) per 10 cm dish of cells. The total amount of DNA in each transfection was normalized using empty vector DNA (pMT2).

Antibodies - DEP-1 monoclonal antibodies A3 and 143-41 used for immunoprecipitations were generous gifts from Dr. Gregorio Aversa and Dr. Antoni Gaya, respectively (Palou et al., *supra*; Tangye et al., *supra*). The DEP-1 polyclonal antibody CS895A was generated against the DEP-1 extracellular domain peptide (CDASNTERSRAGSPTAP, SEQ ID NO: 19) corresponding to amino acids 292-307 coupled to KLH (Pierce, Rockford, IL). The Met polyclonal antibody 144 used for

immunoprecipitations was generated against a carboxy-terminal peptide (Rodrigues et al., *Mol. Cell Biol.* 11:2962-70). The anti-phosphotyrosine monoclonal antibodies G98 and G104 were generated as described (Garton et al., *Mol. Cell Biol.* 16:6408-18 (1996)). Anti-phosphotyrosine-agarose (PT-66) was purchased from Sigma (St. Louis, MO) and anti-phosphotyrosine (4G10) agarose conjugate was purchased from Upstate Biotechnology (Lake Placid, NY). The Met antibody C-12 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies for $p120^{ctn}$, E-cadherin, Grb2, and phosphotyrosine (PY20) were purchased from BD Transduction Labs (Lexington KY). Antibodies specific for β-catenin (6F9) and plakoglobin (15F11) were purchased from Sigma (St. Louis, MO), and the Gab 1 C-terminal antibody was purchased from Upstate Biotechnology (Lake Placid, NY). Anti-c–Met ($pYpYpY^{1230/1234/1235}$) and (pY^{1365}) antibodies were purchased from BioSource International (Camarillo, CA), and Phospho-Met (Tyr^{1349}) antibody was purchased from Cell Signaling Technology (Beverly, MA).

Substrate trapping - Prior to lysis, T-47D and T-47D/Met cells were treated with 50 μM pervanadate for 20 minutes, whereas MDA-MB -231 cells were treated with 100 μM pervanadate for 20 minutes. Cells were rinsed with PBS and lysed in 1% NP-40 buffer (1% NP-40, 150 mM NaCl, 20 mM HEPES pH7.5, 1 mM EDTA, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 1 mM benzamidine). For trapping experiments in vitro, the lysis buffer also contained 5 mM iodoacetic acid to inhibit cellular PTPs irreversibly. After incubation on ice for 5 minutes, dithiothreitol was added to a final concentration of 10 mM to inactivate any unreacted iodoacetic acid. Insoluble material was removed by centrifugation. T-47D lysate (1 mg) or MDA-MB-231 lysate (5 mg) was mixed with MBP or the MBP-DEP-1 constructs bound to amylose resin at a ratio of 1 μg fusion to 500 μg lysate. Lysates and fusion proteins were incubated at 4°C for 2 hours and then washed extensively with 1 % NP-40 buffer. Tyrosine phosphorylated proteins were immunoprecipitated using 0.1 mg T-47D cell lysate and a combination of 5 μl each of anti -phosphotyrosine antibodies PT-66 and 4G10. Lysate and antibodies were incubated at 4°C for 2 hours and washed extensively with 1 % NP-40 buffer. Protein complexes were released by incubation in reducing Laemmli SDS-PAGE

sample buffer at 95°C, subjected to SDS -PAGE on 8% gels, and transferred onto Immobilon-P membranes (Millipore, Bedford, MA) for immunoblotting.

In order to determine whether the tyrosine phosphorylated proteins bound to the substrate -trapping mutants at the PTP active site, the effects of vanadate on complex formation were tested. MBP fusion proteins bound to amylose were pre-incubated in 1 % NP-40 buffer (without EDTA) with or without 2 mM vanadate. Cells were rinsed with PBS and lysed in 1 % NP-40 buffer (without EDTA) with or without 2 mM vanadate. For vanadate competition experiments, the lysis buffer also contained 5 mM iodoacetic acid. After 5 minutes on ice, dithiothreitol was added to a final concentration of 10 mM. Insoluble material was removed by centrifugation, and samples were processed as described above.

Proteins bound to the DEP-1 substrate -trapping mutant were analyzed by immunoblotting. T-47D and T-47D/Met cells were treated and lysed as above. Lysates (30 mg) were mixed with MBP.DEP-1 or MBP-DEP-1(DA) bound to amylose resin at a ratio of 1 μg fusion protein to 500 μg lysate. Lysates and fusion proteins were incubated at 4°C for 2 hours and then washed extensively with 1% NP-40 buffer. Protein complexes were released by incubation in reducing Laemmli SDS-PAGE sample buffer at 95°C, subjected to SDS -PAGE on 8% gels, and transferred onto Immobilon -P membranes for immunoblotting. The samples were divided into 5 mg lysate equivalents per fusion per lane.

Immunoprecipitations - Transfected cells were rinsed with PBS and lysed in 1% NP-40 buffer (1% NP-40, 150 mM NaCl, 20 mM HEPES pH7.5, 1 mM EDTA, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 1 mM benzamadine, 50 mM NaF, 5 mM iodoacetic acid) and processed as above. For substrate-trapping experiments, DEP-1 was immunoprecipitated from 1 mg lysate with the DEP-1 antibodies, A3 and 143–41, and Met was immunoprecipitated from 1 mg of cell lysate using the Met antibody 144.

For dephosphorylation and recruitment experiments, transfected cells were rinsed with PBS and lysed in 1% NP-40 buffer (1% NP-40, 150 mM NaCl, 20 mM HEPES pH 7.5, 1 mM EDTA, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 1 mM benzamadine, 50 mM NaF, 5 mM iodoacetic acid, 1 mM vanadate) and processed as above. Met was immunoprecipitated from 1 mg lysate using the Met antibody 144. Lysate and antibody were incubated at 4°C for

1 hour. Protein A Sepharose 4 Fast Flow (Amersham Pharmacia Biotech, Uppsala, Sweden) was added for 45 minutes at 4°C. Immune complexes were washed extensively with 1% NP-40 buffer, released by incubation in reducing Laemmli SDS-PAGE sample buffer at 95°C, subjected to SDS-PAGE on 8% gels, and transferred onto Immobilon-P membranes for immunoblotting.

EXAMPLE 2

INTERACTION OF A DEP-1(DA) SUBSTRATE-TRAPPING MUTANT WITH A SUBSET OF TYROSINE PHOSPHORYLATED PROTEINS FROM TWO HUMAN BREAST TUMOR LINES

Two human breast tumor lines (T-47D and MDA-MB-23 1), which express DEP-1, were used for in vitro studies to identify potential physiological substrates of the PTP. The cells were treated with pervanadate to generate the broadest spectrum of potential phosphotyrosine containing substrates for analysis. DEP-1 fusion proteins comprising the maltose binding protein (MBP) fused to the N-terminus of the DEP-1 cytoplasmic domain (amino acids 997-1337) were generated. Wild type DEP-1 (MBP.DEP-1), catalytically inactive (MBP.DEP-1(CS)) and substrate-trapping (MBP.DEP-1(DA)) mutant forms of DEP-1 were used for purification of potential substrates by affinity chromatography. T-47D cells were treated with 50 μM pervanadate for 20 minutes prior to cell lysis. DEP-1 fusion proteins were incubated with lysate of pervanadate treated T-47D cells. Tyrosine phosphorylated proteins that interacted with the fusion proteins were visualized by immunoblotting with anti-phosphotyrosine antibodies. The results are presented in Figure 1A. Only the substrate-trapping mutant form of DEP-1 (MBP-DEP-1(DA)) bound tyrosine phosphorylated proteins. In addition, when a comparison was made between the tyrosine phosphorylated proteins that bound to the DEP-1 substrate-trapping mutant and the proteins immunoprecipitated with anti-phosphotyrosine antibodies, MBP-DEP-1(DA) recognized only a small subset of the tyrosine phosphorylated proteins from the lysate of pervanadate treated T-47D cells (Figure 1A).

To determine whether the proteins that interacted with MBP-DEP-1(DA) were potential substrates, the fusion proteins were pre-incubated with vanadate.. Vanadate is a

competitive inhibitor that blocks the PTP active site and prevents substrate binding and phosphatase activity (Huyer et al., *J. Biol. Chem.* 272:843-51 (1997)). Cells were lysed in lysis buffer (see Material and Methods) with (+) or without (-) 2 mM vanadate. MBP and MBP.DEP-1 fusion proteins were pre-incubated with (+) or without (-) 2 mM vanadate and added to cell lysates. Protein complexes were analyzed by SDS -PAGE and immunoblotting using anti-phosphotyrosine antibodies (see Example 1). The immunoblot results are presented in Figure 1B. The interaction between the tyrosine phosphorylated proteins and MBP-DEP-1(DA) was inhibited by vanadate, suggesting that they bound to the active site and may represent substrates of DEP-1.

Similarly, DEP-1 fusion proteins were incubated with the lysate of pervanadate treated MDA-MB-231 cells. MDA-MB-231 cells were treated with 100 μM pervanadate for 20 minutes prior to lysis. MBP or MBP.DEP-1 fusion proteins (MBP.DEP-1, MBP.DEP-1 (CS), MBP.DEP-1 (DA)) were incubated with cell lysates, and protein complexes were analyzed by SDS-PAGE and immunoblotting using anti-phosphotyrosine antibodies. As was observed with the T-47D cell lysates, only the substrate-trapping mutant form of DEP-1 (MBP-DEP-1(DA)) interacted with tyrosine phosphorylated proteins from MDA-MB-231 cell lysates (Figure 2A). Only a small subset of the pool of available tyrosine phosphorylated proteins was recognized by the PTP. MDA-MB-231 cells were treated with 100 μM pervanadate for 20 minutes and then lysed in lysis buffer (see Example 1) with or without 2 mM vanadate. MBP and MBP.DEP-1 fusion proteins were pre-incubated with or without 2 mM vanadate and then added to the cell lysates. The protein complexes that formed were analyzed by SDS-PAGE and immunoblotting using anti-tyrosine antibodies (see Example 1). The immunoblot presented in Figure 2B illustrates that the interaction of the substrate-trapping mutant form of DEP-1 (MBP-DEP-1(DA)) with tyrosine phosphorylated proteins (Figure 1B) was also inhibited by vanadate. Pervanadate treatment resulted in the accumulation of tyrosine phosphorylated proteins in both T-47D and MDA-MB-231 cell lines.

EXAMPLE 3

IDENTIFICATION OF PROTEINS THAT INTERACTED WITH THE DEP-1 SUBSTRATE -TRAPPING MUTANT

Although the tyrosine-phosphorylated proteins that interacted with MBP.DEP-1(DA) were easily detected by immunoblotting with anti-phosphotyrosine antibodies, these proteins were difficult to detect on Coomassie stained gels, suggesting that they were not abundant proteins. From a large-scale preparation of DEP-1 substrates from T-47D cells, cell lysates were prepared and subjected to affinity chromatography using the substrate-trapping mutant form of DEP-1 coupled to the affinity matrix. The bound fraction was separated by SDS-PAGE. On Coomassie stained gels, a 100 kDa protein was detected that corresponded to a 100 kDa tyrosine phosphorylated protein that was detected by immunoblotting (see Figure 1A, arrow). The protein band of apparent Mr 100 kDa was excised from the SDS-PAGE gel. Peptides derived from this protein were sequenced by mass spectrometry according to methods known in the art. Two individual peptides (NLSYQVHR, SEQ ID NO: 20; SQSSHSYDDSTLPLIDR, SEQ ID NO: 21) matched sequences in the src substrate and adherens junction component, $p120^{ctn}$ (Table 1). Both sequences can be found in all the $p120^{ctn}$ isoforms identified to date (*see* Keirsebilck et al., *Genomics* 50:129-46 (1998)). The table presents the peptide sequences and their positions within the various isoforms of $p120^{ctn}$.

Table 1

Identification of p120^{ctn} as a substrate of DEP-1

p120^{ctn} isoform	Peptide sequence and positions of matching amino acids in p120^{ctn} isoforms		GenBank Accession Number
	NLSYQVHR (SEQ ID NO: 20)	SQSSHSYDDSTLPLIDR (SEQ ID NO: 21)	
1ABC	585-592	859-875	AF062321, AF062317
2ABC	531-538	805-821	AF062319
3AB	484-491	752-768	AF062338
4ABC	262-269	536-552	AF062342

Interaction of DEP-1 with other functional components was investigated. T-47D and T-47D/MET cells were cultured as described in Example 1 and were then treated with 50 μM pervanadate for 20 minutes prior to lysis. MBP.DEP-1 or MBP.DEP-1(DA) fusion proteins were incubated with cell lysates. Protein complexes were analyzed by SDS-PAGE and immunoblotting using antibodies directed towards E-cadherin (E-cad), β-catenin (Beta-cat), plakoglobin (Pg), p120^{ctn} (p120), Met (Met) and Gab 1 (Gab 1) (see Example 1). Total cell lysate was analyzed to confirm the expression and molecular weight of each of the proteins identified by immunoblotting. As shown in Figure 2, immunoblot analysis revealed that the DEP-1 substrate-trapping mutant (DA) did not interact with the transmembrane protein E-cadherin from pervanadate treated T-47D cell lysates. The cytoplasmic proteins β-catenin and plakoglobin, however, were found in a complex with MBP.DEP-1(DA). Although p120^{ctn} only interacted with the DEP-1 substrate-trapping mutant, β-catenin and plakoglobin also interacted with the wild type form of the enzyme (MBP-DEP-1) (Figure 3).

As discussed above, the DEP-1 substrate-trapping mutant bound several tyrosine-phosphorylated proteins from both T-47D and MDA-MB-231 cell lines (see Figure 1, Figure 2). On the basis of the molecular weights of these proteins and the observation that

DEP-1 interacted with components of adherens junctions, experiments were conducted to probe for signaling molecules known to localize to cell-cell junctions. MBP.DEP-1(DA) trapped Met, the HGF/SF receptor, from pervanadate-treated MDA-MB-231 cells (data not shown). Since Met is expressed at low levels in T-47D cells, a T-47D stable cell line ectopically expressing the PTK (T-47D/Met) was employed, which has been used previously in analysis of Met function (Shen et al., *supra*). MBP-DEP-1(DA) also trapped Met from pervanadate treated T-47D/Met cell lysate, and this interaction was not observed between the wild type DEP-1 (MBP-DEP-1) and Met (Figure 3). This suggested a transient interaction between DEP-1 and Met, which is consistent with that of enzyme and substrate.

MBP-DEP-1(DA) trapped the docking protein Gab 1 from T-47D/Met cell lysates (Figure 3), which is consistent with earlier reports of pleiotropic effects mediated by Met through recruitment of a number of docking and signaling molecules (reviewed in Furge et al., *supra*). Following activation of Met, Gab 1 was reported to be recruited to the kinase and phosphorylated on tyrosine residues, permitting recruitment of other signaling and adapter molecules, thereby amplifying downstream signals. As shown in Figure 3, MBP.DEP-1(DA) also trapped Gab 1 from T-47D cells suggesting that the Gab 1-DEP-1 interaction is at least partially direct in a manner that does not require Met.

EXAMPLE 4

INTERACTION BETWEEN FULL LENGTH DEP-1(DA) SUBSTRATE -TRAPPING MUTANT AND MET (HGF-R/SF-R) FROM 293 CELLS

As noted above, full length DEP-1 is a transmembrane PTP. As shown in the preceding Examples, however, by using only the cytoplasmic domain of the substrate-trapping mutant DEP-1, an interaction of DEP-1 with Met was observed. To determine whether the trapping mutant form of full length DEP-1 also trapped Met, each of full length DEP-1 and the mutants DEP-1 (CS) and DEP-1(DA) was co-expressed with a chimeric Met construct CSF-MET. This chimeric receptor, which comprised the extracellular domain of human colony stimulating factor 1 receptor (CSF-1R) and the transmembrane and cytoplasmic domains of

human Met (Zhu et al., *supra*), was constitutively active when expressed in 293 cells, bypassing the requirement for ligand stimulation. 293 cells were tranfected with CSF-MET alone or in combination with wild type or mutant forms of DEP-1. Cells were serum-starved and then cell lysates were prepared as described in Example 1. The wild type DEP-1 and the DEP-1 mutants were immunoprecipitated from half of the cell lysates using monoclonal antibodies, A3 and 143-41 under conditions that preserved protein complexes. The immunoprecipitates were then separated by SDS-PAGE and transferred to Immobilon P membranes for immunoblotting. The levels of wild type DEP-1 and the DEP-1 (DA) and DEP-1 (CS) were determinined by probing the immunoblots with the polyclonal antibody CS895A. The immunoblots were then stripped and reprobed for Met. The results are presented in Figure 4A. Similar levels of DEP-1, DEP-1(CS), and DEP-1(DA) were immunoprecipitated from 293 cell lysates (Figure 4A). No endogenous DEP-1 could be detected in immunoprecipitates from 293 cells expressing the Met chimera alone. As with the DEP-1(DA) cytoplasmic domain fusion protein, full length DEP-1(DA) formed a stable complex with Met (Figure 4A). The full length DEP-1(CS) mutant also bound Met, but less efficiently than the DEP-1(DA) mutant. Similar results were observed in the interaction between PTP-PEST and its substrate $p130^{cas}$ (Garton et al., *supra*). No stable interaction was observed between wild type DEP-1 and Met when co-expressed in 293 cells (Figure 4A, second lane).

Full-length wild type DEP-I was also observed to dephosphorylate Met in 293 cells. Because full-length substrate-trapping mutant forms of DEP-1 bound Met when co-expressed in 293 cells (Figure 4A), whether full-length wild type DEP-1 could dephosphorylate Met was investigated. Full-length DEP-1 and the mutants DEP-1 (CS) and DEP-1(DA) were co-expressed with the CSF-MET chimera in 293 cells as described above. The Met chimera was immunoprecipitated from cell lysates with an antibody specific for the Met portion of the chimera. As shown in Figure 4B, immunoblots revealed that similar levels of CSF-MET were immunoprecipitated in each condition. The Met chimera was tyrosine phosphorylated when it was expressed alone in 293 cells; however, the presence of tyrosine phosphorylation was not detected when it was co-expressed with wild type DEP-1 (Figure 4B,

lane 2, lower immunoblot). Although the DEP-1(CS) and DEP-1 (DA) mutants interacted with the Met chimera (Figure 4A), Met was not dephosphorylated in the cells expressing these mutants, suggesting that dephosphorylation required DEP-1 catalytic activity.

EXAMPLE 5

PREFERENTIAL DEPHOSPHORYLATION OF C-TERMINAL PHOSOPHOTYROSINE RESIDUES IN MET BY DEP-1

When equal amounts of wild type DEP-1 and CSF-MET plasmid DNA were transfected into 293 cells, the level of DEP-1 protein expressed was sufficient to dephosphorylate Met (Figure 4B). A dose-response analysis was performed to determine whether varying the expression level of DEP-1 would affect its ability to dephosphorylate Met. 293 cells were transfected with a constant concentration of CSF-MET DNA (20 μg) and increasing amounts of wild type DEP-1 DNA (0, 1, 2.5, 5, 10 μg) or 10 μg of the catalytically inactive DEP-1(CS) mutant DNA (Figure 5A). Immunoblots showed that as the levels of DEP-1 plasmid DNA used for transfection were increased, the level of DEP-1 protein that was expressed also increased, whereas the levels of Met protein detected were similar, independent of the level of DEP-1 expressed (Figure 5A).

Dephosphorylation by DEP-1 of specific tyrosine residues of the Met polypeptide was examined. Met was immunoprecipitated from the lysates of serum-starved 293 cells prepared as described above using the polyclonal antibody 144. The immunoprecipitates were separated by SDS-PAGE in duplicate and transferred to membranes for immunoblotting as described in Example 1. An immunoblots was probed with the polyclonal antibody C-12 as shown in Figure 5B, which revealed a constant level of Met immunoprecipitated from the cell lysates (MET). This blot was stripped and re-probed with the phospho-specific antibody to Tyr^{1349} in Met (Phospho-Met Y^{1349}). A duplicate blot was probed with anti-phosphotyrosine antibodies to illustrate the total phosphotyrosine content (PY), then sequentially stripped and re-probed with phospho-specific antibodies to examine the phosphorylation status of Tyr^{1230}, Tyr^{1234} and Tyr^{1235} (Phospho-Met $Y^{1230/34/35}$), and Tyr^{1365}

(phospho-Met Y^{1365}). Although similar amounts of Met were immunoprecipitated from 293 cell lysates, a gradual decrease in the level of phosphorylation of Met was detected with increasing expression of wild type DEP-1 (Figure 5B). The phosphorylation of Met when Met was expressed alone was similar with the phosphorylation of Met when expressed with the catalytically inactive form of DEP-1 (DEP-1(CS)).

Met contains three tyrosines in the activation loop of the catalytic domain (Tyr^{1230}, Tyr^{1234} and Tyr^{1235}), and phosphorylation of Tyr^{1234} and Tyr^{1235} is required for full activation of the kinase (Rodrigues et al., *Oncogene* 9:2019-27 (1994)). To determine whether DEP-1 acted on these tyrosine residues, phospho-specific antibodies were employed. Met was immunoprecipitated from the lysates of serum-starved 293 cells (see above) using the polyclonal antibody 144. Duplicate samples of mmunoprecipitates were separated by SDS-PAGE and immunoblotted. Immunoblots probed with the polyclonal antibody C-12 revealed a constant level of Met immunoprecipitated from the cell lysates (MET). This blot was stripped and re-probed with the phospho-specific antibody to Tyr^{1349} in Met (Phospho-Met Y^{1349}). A duplicate blot was probed with anti-phosphotyrosine antibodies to illustrate the total phosphotyrosine content, then sequentially stripped and re-probed with phospho-specific antibodies to examine the phosphorylation status of Tyr^{1230}, Tyr^{1234}, and Tyr^{1235} (Phospho-Met $Y^{1230/34/35}$), and Tyr^{1365} (phospho-Met Y^{1365}). Figure 5B shows that similar to the effects on the overall levels of Met phosphorylation, a gradual decrease in the level of phosphorylation of the activation loop tyrosine residues was observed with increasing expression of wild type DEP–1, and no effect on phosphorylation of Met was observed with the expression of DEP-1(CS). Phosphorylation of Tyr^{1349} and Tyr^{1356} in the multi -substrate docking site of Met was required for the transduction of downstream signals: Tyr^{1349} was previously shown to be a binding site for the adapter protein Gab 1, whereas Tyr^{1356} was primarily responsible for binding Grb2, PI3K, PLC -γ and SHP2 (reviewed in Furge et al., *supra*). Phospho-specific antibodies towards Tyr^{1349} were used to determine whether DEP-1 dephosphorylated this site. In contrast to the gradual reduction in phosphorylation that was seen for the activation loop tyrosine residues, Tyr^{1349} was nearly completely dephosphorylated in the presence of low levels of DEP-1 (Figure 5B). This dephosphorylation also required DEP-1 catalytic activity since no

change in the phosphorylation level of Tyr^{1349} was observed in the presence of DEP-1(CS). In addition to the docking site tyrosine residues, other tyrosine residues have been shown to impact Met signaling. For example, Tyr^{1365} was important for mediating a morphogenic signal (Weidner et al., (1995), *supra*). Phospho-specific antibodies directed towards this site revealed that Tyr^{1365} was nearly completely dephosphorylated in the presence of low levels of DEP-1 (Figure 5B).

EXAMPLE 6

EFFECTS OF INCREASED DEP-1 EXPRESSION ON THE INTERACTION BETWEEN MET AND GRB2

Ligand-induced activation of Met resulted in the recruitment of a number of proteins that were important for transmitting downstream signals. The dephosphorylation of a docking site tyrosine residue in Met, as detected in the preceding Examples, prompted examination of the recruitment of Grb2. Met was immunoprecipitated as described in Example 5 from serum-starved 293 cells co-expressing CSF-MET. Varying amounts of DEP-1 and the immunoprecipitates were probed for the presence of the Grb2 adapter protein. Grb2 was previously reported to bind to Met directly via Tyr^{1365} (Fixman et al., *supra*; Ponzetto et al. (1994), *supra*). Immunoblots of cell lysates and MET-immunoprecipitates probed with an antibody specific for Grb2 (BD Transduction Labs) revealed that the level of Grb2 was not affected by the expression of DEP-1 and Met in these cells (Figure 5C, lower blot). However, with increasing levels of DEP-1 a gradual decrease in the amount of Grb2 that co-immunoprecipitated with Met was observed (Figure 5C, upper blot) coincident with the changes in overall tyrosine phosphorylation status of the PTK.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.